P&G Case 7998

COMPOSITIONS AND METHODS FOR TREATING HAIR LOSS USING NON-NATURALLY OCCURRING PROSTAGLANDINS

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FIELD OF THE INVENTION

This invention relates to compositions and methods for treating hair loss in mammals. More particularly, this invention relates to compositions and methods for arresting or reversing hair loss, or both, and promoting hair growth.

BACKGROUND OF THE INVENTION

Hair loss is a common problem which is, for example, naturally occurring or chemically promoted through the use of certain therapeutic drugs designed to alleviate conditions such as cancer. Often such hair loss is accompanied by lack of hair re-growth which causes partial or full baldness.

Hair growth on the scalp does not occur continuously, but rather occurs by a cycle of activity involving alternating periods of growth and rest. This cycle is divided into three main stages; anagen, catagen, and telogen. Anagen is the growth phase of the cycle and is characterized by penetration of the hair follicle deep into the dermis with rapid proliferation of cells which are differentiating to form hair. The next phase is catagen,

which is a transitional stage marked by the cessation of cell division, and during which the hair follicle regresses through the dermis and hair growth ceases. The next phase, telogen, is characterized as the resting stage during which the regressed follicle contains a germ with tightly packed dermal papilla cells. At telogen, the initiation of a new anagen phase is caused by rapid cell proliferation in the germ, expansion of the dermal papilla, and elaboration of basement membrane components. When hair growth ceases, most of the hair follicles reside in telogen and anagen is not engaged, thus causing the onset of full or partial baldness.

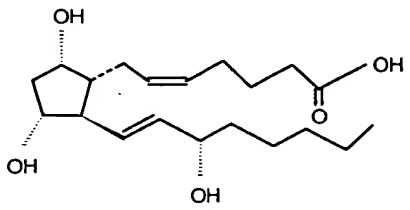
Attempts to invoke the re-growth of hair have been made by, for example, the promotion or prolongation of anagen. Currently, there are two drugs approved by the United States Food and Drug Administration for the treatment of male pattern baldness: topical minoxidil (marketed as ROGAINE® by Pharmacia & Upjohn), and oral finasteride (marketed as PROPECIA® by Merck & Co., Inc.). However, the search for efficacious hair growth inducers is ongoing due to factors including safety concerns and limited efficacy.

The thyroid hormone thyroxine ("T4") converts to thyronine ("T3") in human skin by deiodinase I, a selenoprotein. Selenium deficiency causes a decrease in T3 levels due to a decrease in deiodinase I activity; this reduction in T3 levels is strongly associated with hair loss. Consistent with this observation, hair growth is a reported side effect of administration of T4. See, e.g., Berman, "Peripheral Effects of L-Thyroxine on Hair Growth and Coloration in Cattle", Journal of Endocrinology, Vol. 20, pp. 282 - 292 (1960); and Gunaratnam, "The Effects of Thyroxine on Hair Growth in the Dog", J. Small Anim. Pract., Vol. 27, pp. 17 - 29 (1986). Furthermore, T3 and T4 have been the subject of several patent publications relating to treatment of hair loss. See, e.g., Fischer et al., DE 1,617,477, published January 8, 1970; Mortimer, GB 2,138,286, published October 24, 1984; and Lindenbaum, WO 96/25943, assigned to Life Medical Sciences, Inc., published August 29, 1996.

Unfortunately, however, administration of T3 or T4, or both, to treat hair loss is often not practicable because these thyroid hormones can induce significant cardiotoxicity. See, e.g., Walker et al., U.S. Patent No. 5,284,971, assigned to Syntex,

issued February 8, 1994 and Emmett et al., U.S. Patent No. 5,061,798, assigned to Smith Kline & French Laboratories, issued October 29, 1991.

In an alternative approach, prostaglandins have been proposed to promote hair growth because prostaglandins may have a similar benefit to thyroid hormones, i.e., increasing hair length and changing pigmentation. Naturally occurring prostaglandins (e.g., PGA_2, PGB_2, PGE_1, $PGF_{2\alpha}$, and PGI_2) are C-20 unsaturated fatty acids. $PGF_{2\alpha}$, the naturally occurring Prostaglandin F analog in humans, is characterized by hydroxyl groups at the C9 and C11 positions on the alicyclic ring, a cis-double bond between C5 and C6, and a trans-double bond between C13 and C14. $PGF_{2\alpha}$ has the formula:



Analogs of naturally occurring Prostaglandin F are known in the art. For example, see U.S. Patent No. 4,024,179 issued to Bindra and Johnson on May 17, 1977; German Patent No. DT-002,460,990 issued to Beck, Lerch, Seeger, and Teufel published on Jul. 1, 1976; U.S. Patent No. 4,128,720 issued to Hayashi, Kori, and Miyake on Dec. 5, 1978; U.S. Patent No. 4,011,262 issued to Hess, Johnson, Bindra, and Schaaf on Mar. 8, 1977; U.S. Patent No. 3,776,938 issued to Bergstrom and Sjovall on Dec. 4, 1973; P. W. Collins and S. W. Djuric, "Synthesis of Therapeutically Useful Prostaglandin and Prostacyclin Analogs", Chem. Rev., Vol. 93, pp. 1533-1564 (1993); G. L. Bundy and F. H. Lincoln, "Synthesis of 17-Phenyl-18,19,20-Trinorprostaglandins: I. The PG_1 Series", Prostaglandin, Vol. 9 No. 1, pp. 1-4 (1975); W. Bartman, G. Beck, U. Lerch, H. Teufel, and B. Scholkens, "Luteolytic Prostaglandin: Synthesis and Biological Activity", Prostaglandin, Vol. 17 No. 2, pp. 301-311 (1979); C. Iiljebris, G. Selen, B. Resul, J. Sternschantz, and U. Hacksell, "Derivatives of 17-Phenyl-18, 19,20-trinorprostaglandin $F_2\alpha$. Isopropyl Ester: Potential Antiglaucoma Agents", Journal of Medicinal Chemistry, Vol. 38, No. 2, pp. 289-304 (1995).

Prostaglandins in general have a wide range of biological activities. For example, PGE_2 has the following properties: a) regulator of cell proliferation, b) regulator of

cytokine synthesis, c) regulator of immune responses and d) inducer of vasodilatation. Vasodilatation is thought to be one of the mechanisms of how minoxidil provides a hair growth benefit. *In vitro* results in the literature also indicate some anti-inflammatory properties of the prostaglandins. c.f.; Tanaka, H. Br J. Pharm., 116, 2298, (1995).

However, previous attempts at using prostaglandins to promote hair growth have been unsuccessful. Different prostaglandin analogs can bind to multiple receptors at various concentrations with a biphasic effect. Furthermore, administration of naturally occurring prostaglandins can cause side effects such as inflammation, surface irritation, smooth muscle contraction, pain, and bronchoconstriction. Therefore, it is an object of this invention to provide methods for using prostaglandin analogs to grow hair and to provide compositions that promote hair growth in humans and lower animals. It is a further object of this invention to provide a selection of appropriate prostaglandin analogs that will promote hair growth and that do not cause significant undesirable side effects.

SUMMARY OF THE INVENTION

This invention relates to compositions and methods for treating hair loss. The methods comprise administering the compositions comprising specific prostaglandin analogs that interact strongly with hair-selective receptors, such as the FP receptor. The choice of prostaglandin analog is important because the prostaglandin analogs must selectively activate the FP receptor and not activate any other receptors that would negate the effect of activating the FP receptor. The compositions comprise: component A) the prostaglandin analog, component B) a carrier, and optionally component C) an activity enhancer.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to compositions and methods using prostaglandin F analogs ("PGF's") to treat hair loss in mammals. "Treating hair loss" includes arresting hair loss or reversing hair loss, or both, and promoting hair growth.

Publications and patents are referred to throughout this disclosure. All U.S. Patents cited herein are hereby incorporated by reference.

All percentages, ratios, and proportions used herein are by weight unless otherwise specified.

Definition and Usage of Terms

The following is a list of definitions for terms, as used herein:

"Activate" means binding and signal transduction of a receptor.

"Acyl group" means a monovalent group suitable for acylating a nitrogen atom to form an amide or carbamate, an alcohol to form a carbonate, or an oxygen atom to form an ester group. Preferred acyl groups include benzoyl, acetyl, tert-butyl acetyl, para-phenyl benzoyl, and trifluoroacetyl. More preferred acyl groups include acetyl and benzoyl. The most preferred acyl group is acetyl.

"Aromatic group" means a monovalent group having a monocyclic ring structure or fused bicyclic ring structure. Monocyclic aromatic groups contain 5 to 10 carbon atoms, preferably 5 to 7 carbon atoms, and more preferably 5 to 6 carbon atoms in the ring. Bicyclic aromatic groups contain 8 to 12 carbon atoms, preferably 9 or 10 carbon atoms in the ring. Aromatic groups are unsubstituted. The most preferred aromatic group is phenyl. Bicyclic aromatic groups include ring systems wherein one ring in the system is aromatic. Preferred bicyclic aromatic groups are ring systems wherein both rings in the system are aromatic. Preferred aromatic rings include naphthyl and phenyl. The most preferred aromatic ring is phenyl.

"Carbocyclic group" means a monovalent saturated or unsaturated hydrocarbon ring. Carbocyclic groups are monocyclic. Carbocyclic groups contain 4 to 10 carbon atoms, preferably 4 to 7 carbon atoms, and more preferably 5 to 6 carbon atoms in the ring. Carbocyclic groups are unsubstituted. Preferred carbocyclic groups include cyclopentyl, cyclohexyl, cyclohexenyl, cycloheptyl, and cyclooctyl. More preferred carbocyclic groups include cyclohexyl, cycloheptyl, and cyclooctyl. The most preferred carbocyclic group is cycloheptyl. Carbocyclic groups are not aromatic.

"FP agonist" means a compound that activates the FP receptor.

"FP receptor" means known human FP receptors, their splice variants, and undescribed receptors that have similar binding and activation profiles as the known human FP receptors. "FP" means the receptor is of the class which has the highest affinity for $PGF_{2\alpha}$ of all the naturally occurring prostaglandins. FP refers to a known protein.

"Halogen atom" means F, Cl, Br, or I. Preferably, the halogen atom is F, Cl, or Br; more preferably Cl or F; and most preferably F.

"Halogenated heterogenous group" means a substituted heterogenous group or a substituted heterocyclic group, wherein at least one substituent is a halogen atom. Halogenated heterogenous groups can have a straight, branched, or cyclic structure. Preferred halogenated heterogenous groups have 1 to 12 carbon atoms, more preferably 1 to 6 carbon atoms, and most preferably 1 to 3 carbon atoms. Preferred halogen atom substituents are Cl and F.

"Halogenated hydrocarbon group" means a substituted monovalent hydrocarbon group or a substituted carbocyclic group, wherein at least one substituent is a halogen atom. Halogenated hydrocarbon groups can have a straight, branched, or cyclic structure. Preferred halogenated hydrocarbon groups have 1 to 12 carbon atoms, more preferably 1 to 6 carbon atoms, and most preferably 1 to 3 carbon atoms. Preferred halogen atom substituents are Cl and F. The most preferred halogenated hydrocarbon group is trifluoromethyl.

"Heteroaromatic group" means an aromatic ring containing carbon and 1 to 4 heteroatoms in the ring. Heteroaromatic groups are monocyclic or fused bicyclic rings. Monocyclic heteroaromatic groups contain 5 to 10 member atoms (i.e., carbon and heteroatoms), preferably 5 to 7, and more preferably 5 to 6 in the ring. Bicyclic heteroaromatic rings contain 8 to 12 member atoms, preferably 9 or 10 in the ring. Heteroaromatic groups are unsubstituted. Bicyclic heteroaromatic groups include ring systems in which only one ring is aromatic. Preferred bicyclic heteroaromatic groups are ring systems in which both rings are aromatic. Preferred monocyclic heteroaromatic groups include thienyl, thiazolyl, purinyl, pyrimidyl, pyridyl, and furanyl. More preferred monocyclic heteroaromatic groups include thienyl, furanyl, and pyridyl. The most preferred monocyclic heteroaromatic group is thienyl. Preferred bicyclic heteroaromatic

rings include benzothiazolyl, benzothiophenyl, quinolinyl, quinoxalinyl, benzofuranyl, benzimidazolyl, benzoxazolyl, indolyl, and anthranilyl. More preferred bicyclic heteroaromatic rings include benzothiazolyl, benzothiophenyl, and benzoxazolyl.

"Heteroatom" means an atom other than carbon in the ring of a heterocyclic group or the chain of a heterogeneous group. Preferably, heteroatoms are selected from the group consisting of nitrogen, sulfur, and oxygen atoms. Groups containing more than one heteroatom may contain different heteroatoms.

"Heterocyclic group" means a saturated or unsaturated ring structure containing carbon and 1 to 4 heteroatoms in the ring. No two heteroatoms are adjacent in the ring, and no carbon in the ring that has a heteroatom bonded to it also has a hydroxyl, amino, or thiol group bonded to it. Heterocyclic groups are not aromatic. Heterocyclic groups are monocyclic. Heterocyclic groups contain 4 to 10 member atoms (i.e., including both carbon atoms and at least 1 heteroatom), preferably 4 to 7, and more preferably 5 to 6 in the ring. Heterocyclic groups are unsubstituted. Preferred heterocyclic groups include piperzyl, morpholinyl, tetrahydrofuranyl, tetrahydropyranyl, and piperdyl.

"Heterogeneous group" means a saturated or unsaturated chain containing 1 to 18 member atoms (i.e., including both carbon and at least one heteroatom). No two heteroatoms are adjacent. Preferably, the chain contains 1 to 12 member atoms, more preferably 1 to 6.. "Lower heterogeneous" means a heterogeneous group having 1 to 6, preferably 1 to 3, member atoms. The chain may be straight or branched. Preferred branched heterogeneous groups have one or two branches, preferably one branch. Preferred heterogeneous groups are saturated. Unsaturated heterogeneous groups have one or more double bonds, one or more triple bonds, or both. Preferred unsaturated heterogeneous groups have one or two double bonds or one triple bond. More preferably, the unsaturated heterogeneous group has one double bond. Heterogeneous groups are unsubstituted.

"Monovalent hydrocarbon group" means a chain of 1 to 18, preferably 1 to 12, carbon atoms. "Lower monovalent hydrocarbon group" means a monovalent hydrocarbon group having 1 to 6, preferably 1 to 3, carbon atoms. Monovalent hydrocarbon groups may have a straight chain or branched chain structure. Preferred monovalent hydrocarbon

groups have one or two branches, preferably 1 branch. Preferred monovalent hydrocarbon groups are saturated. Unsaturated monovalent hydrocarbon groups have one or more double bonds, one or more triple bonds, or combinations thereof. Preferred unsaturated monovalent hydrocarbon groups have one or two double bonds or one triple bond; more preferred unsaturated monovalent hydrocarbon groups have one double bond.

"Pharmaceutically acceptable" means suitable for use in a human or other mammal.

"Prostaglandin" means a fatty acid derivative which has a variety of potent biological activities of a hormonal or regulatory nature.

"Protecting group" is a group that replaces the active hydrogen of a hydroxyl moiety thus preventing undesired side reaction at the hydroxyl moiety. Use of protecting groups in organic synthesis is well known in the art. Examples of protecting groups are found in Chapter 2 Protecting Groups in Organic Synthesis by Greene, T. W. and Wuts, P. G. M., 2nd ed., Wiley & Sons, Inc., 1991. Preferred protecting groups include silyl ethers, alkoxymethyl ethers, tetrahydropyranyl, tetrahydrofuranyl, esters, and substituted or unsubstituted benzyl ethers.

"Safe and effective amount" means a quantity of a prostaglandin high enough to provide a significant positive modification of the subject's condition to be treated, but low enough to avoid serious side effects (at a reasonable benefit/risk ratio).

"Selective" means having a binding or activation preference for a specific receptor over other receptors which can be quantitated based upon receptor binding or activation assays.

"Subject" means a living, vertebrate, hair- or fur-bearing animal such as a mammal (preferably human) in need of treatment.

"Substituted aromatic group" means an aromatic group wherein 1 to 4 of the hydrogen atoms bonded to carbon atoms in the ring have been replaced with other substituents. Preferred substituents include: halogen atoms, cyano groups, monovalent hydrocarbon groups, substituted monovalent hydrocarbon groups, heterogeneous groups, substituted heterogeneous groups, aromatic groups, substituted aromatic groups, or any combination thereof. More preferred substituents include halogen atoms, halogenated

monovalent hydrocarbon groups, phenyl groups, and phenoxy groups. Preferred substituted aromatic groups include naphthyl. The substituents may be substituted at the ortho, meta, or para position on the ring, or any combination thereof. The preferred substitution pattern on the ring is ortho or meta. The most preferred substitution pattern is ortho.

"Substituted carbocyclic group" means a carbocyclic group wherein 1 to 4 hydrogen atoms bonded to carbon atoms in the ring have been replaced with other substituents. Preferred substituents include: halogen atoms, cyano groups, monovalent hydrocarbon groups, monovalent heterogeneous groups, substituted monovalent hydrocarbon groups, substituted heterogeneous groups, aromatic groups, substituted aromatic groups, or any combination thereof. More preferred substituents include halogen atoms, halogenated monovalent hydrocarbon groups, phenyl groups, and phenoxy groups.

"Substituted heteroaromatic group" means a heteroaromatic group wherein 1 to 4 hydrogen atoms bonded to carbon atoms in the ring have been replaced with other substituents. The substituents include halogen atoms, acyl groups, cyano groups, monovalent hydrocarbon groups, substituted monovalent hydrocarbon groups, heterogeneous groups, substituted heterogeneous groups, aromatic groups, substituted aromatic groups, heteroaromatic groups, substituted heteroaromatic groups, and any combination thereof. Preferred substituents include halogen atoms, cyano groups, monovalent hydrocarbon groups, substituted monovalent hydrocarbon groups, heterogeneous groups, substituted heterogeneous groups, phenyl groups, phenoxy groups, or any combination thereof. More preferred substituents include halogen atoms, halogenated hydrocarbon groups, monovalent hydrocarbon groups, halogenated heterogenous groups, and phenyl groups.

"Substituted heterocyclic group" means a heterocyclic group wherein 1 to 4 hydrogen atoms bonded to carbon atoms in the ring have been replaced with other substituents. Preferred substituents include: halogen atoms, cyano groups, monovalent hydrocarbon groups, substituted monovalent hydrocarbon groups, heterogeneous groups, substituted heterogeneous groups, aromatic groups, substituted aromatic groups, or any combination thereof. More preferred substituents include halogen atoms, halogenated

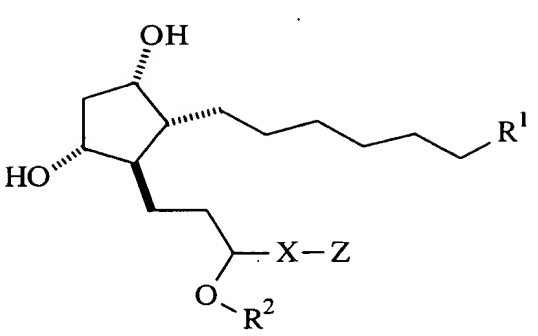
hydrocarbon groups, phenyl groups, phenoxy groups, or any combination thereof. Substituted heterocyclic groups are not aromatic.

"Substituted heterogeneous group" means a heterogeneous group, wherein 1 to 4 of the hydrogen atoms bonded to carbon atoms in the chain have been replaced with other substituents. Preferably substituted heterogeneous groups are mono, di, or trisubstituted. Preferred substituents include halogen atoms, hydroxy groups, carboxy groups, aryloxy groups (e.g., phenoxy, chlorophenoxy, tolyloxy, methoxyphenoxy, benzyloxy, alkyloxycarbonylphenoxy, and acyloxyphenoxy), acyloxy groups (e.g., propionyloxy, benzoyloxy, and acetoxy), aromatic groups (e.g., phenyl and tolyl), substituted aromatic groups (e.g., alkoxyphenyl, alkoxycarbonylphenyl, and halophenyl), heterocyclic groups, heteroaromatic groups, substituted heterocyclic groups, and amino groups (e.g., amino, mono- and di- alkylamino having 1 to 3 carbon atoms, methylphenylamino, methylbenzylamino, alkanylamido groups of 1 to 3 carbon atoms, carbamamido, ureido, and guanidino).

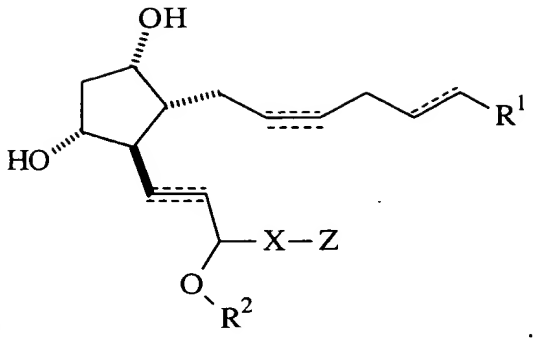
"Substituted monovalent hydrocarbon group" means a monovalent hydrocarbon group wherein 1 to 4 of the hydrogen atoms bonded to carbon atoms in the chain have been replaced with other substituents. Preferred substituted monovalent hydrocarbon groups are mono, di, or trisubstituted. Preferred substituents include halogen atoms; lower monovalent hydrocarbon groups; hydroxy groups; aryloxy groups (e.g., phenoxy, chlorophenoxy, tolyloxy, methoxyphenoxy, benzyloxy, alkyloxycarbonylphenoxy, and acyloxyphenoxy); acyloxy groups (e.g., propionyloxy, benzoyloxy, and acetoxy); carboxy groups; monocyclic aromatic groups; monocyclic heteroaromatic groups; monocyclic carbocyclic groups, monocyclic heterocyclic groups, and amino groups (e.g., amino, mono- and di- alkanylamino groups of 1 to 3 carbon atoms, methylphenylamino, methylbenzylamino, alkanylamido groups of 1 to 3 carbon atoms, carbamamido, ureido, and guanidino).

Prostaglandins Used in the Invention

This invention relates to the use of prostaglandin F analogs (PGF's) to treat hair loss. Suitable PGF's can have a structure selected from the group consisting of:



and



The PGF can also be selected from the group consisting of pharmaceutically acceptable salts and hydrates of the structures above; biohydrolyzable amides, esters, and imides of the structures above; and optical isomers, diastereomers, and enantiomers of the structures above. Thus, at all stereocenters where stereochemistry is not defined (C_{11}, C_{12}, and C_{15}), both epimers are envisioned. Preferred stereochemistry at all such stereocenters of the compounds of the invention mimic that of naturally occurring $PGF_{2\alpha}$. A combination of two or more PGF's can also be used.

R^1 is selected from the group consisting of C(O)OH, C(O)NHOH, $C(O)OR^3$, CH_2OH, $S(O)_2R^3$, $C(O)NHR^3$, $C(O)NHS(O)_2R^4$, tetrazole, a cationic salt moiety, a pharmaceutically acceptable amine or ester comprising 2 to 13 carbon atoms, and a biometabolizable amine or ester comprising 2 to 13 atoms. Preferably, R^1 is selected from the group consisting of CO_2H, C(O)NHOH, CO_2R^3, $C(O)NHS(O)_2R^4$, and tetrazole. More preferably, R^1 is selected from the group consisting of CO_2H and CO_2R^3.

R^2 is selected from the group consisting of a hydrogen atom, a lower heterogenous group, and lower monovalent hydrocarbon groups. Preferably, R^2 is a hydrogen atom.

R^3 is selected from the group consisting of a monovalent hydrocarbon group, a heterogeneous group, a carbocyclic group, a heterocyclic group, an aromatic group, a heteroaromatic group, a substituted monovalent hydrocarbon group, a substituted heterogeneous group, a substituted carbocyclic group, a substituted heterocyclic group, a substituted aromatic group, and a substituted heteroaromatic group. Preferably, R^3 is selected from the group consisting of methyl, ethyl, and isopropyl

R^4 is selected from the group consisting of a monovalent hydrocarbon group, a heterogeneous group, a carbocyclic group, a heterocyclic group, an aromatic group, a heteroaromatic group, a substituted monovalent hydrocarbon group, a substituted

heterogeneous group, a substituted carbocyclic group, a substituted heterocyclic group, a substituted aromatic group, and a substituted heteroaromatic group. Preferably, R^4 is a phenyl group.

X is divalent. X is selected from the group consisting of -C≡C-, a covalent bond, -CH=C=CH-, -CH=CH-, -CH=N-, -C(O)-, -C(O)Y-, $-(CH_2)_n-$, wherein n is 2 to 4, $-CH_2NH-$, $-CH_2S-$, and $-CH_2O-$.

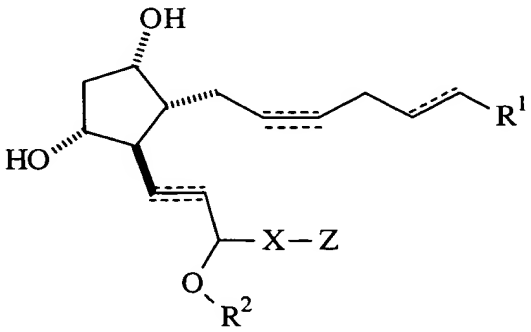
Y is selected from the group consisting of O, S, and NH.

Z is selected from the group consisting of a carbocyclic group, a heterocyclic group, an aromatic group, a heteroaromatic group, a substituted carbocyclic group, a substituted heterocyclic group, a substituted aromatic group, and a substituted heteroaromatic group.

Preferably, when X is a covalent bond, Z is selected from the group consisting of an aromatic group, a heteroaromatic group, a substituted aromatic group, and a substituted heteroaromatic group. More preferably, when X is a covalent bond, Z is a bicyclic heteroaromatic group.

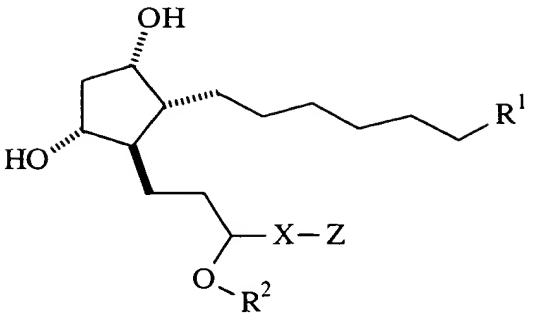
Preferably, when X is -C≡C-, Z is a monocyclic aromatic group. More preferably, when X is -C≡C-, Z is selected from the group consistng of furanyl, thienyl, and phenyl.

Bonds shown as dashed lines in the second structure above indicate that those bonds may optionally be double or triple bonds. For example, when R^1 is C(O)OH in the structure:



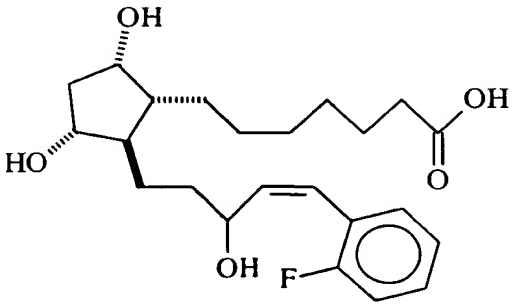
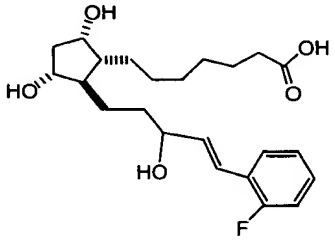
The bond at the C2-C3 position may be a single bond or a double bond. The bond at the C5-C6 position may be a single, double, or triple bond. The bond at the C13-C14 position may be a single, double, or triple bond.

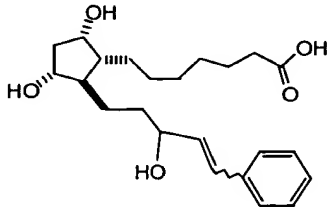
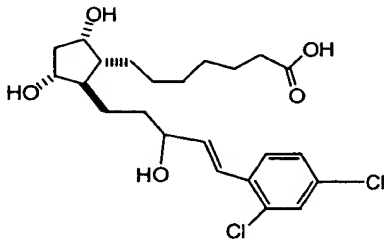
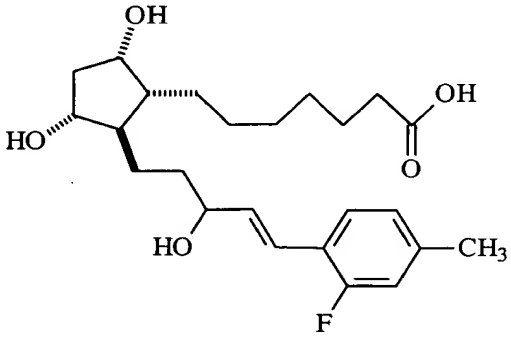
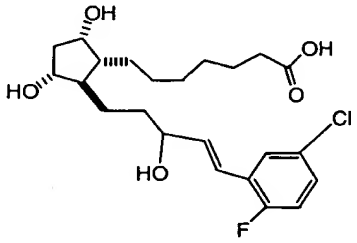
Examples of PGF's' having the structure:

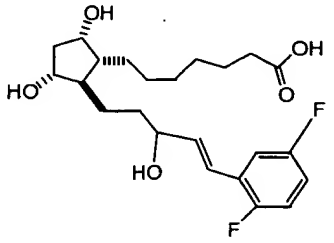
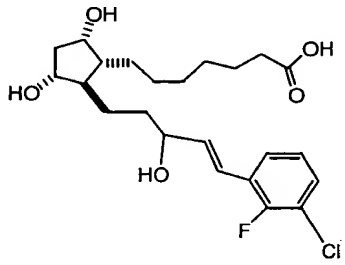
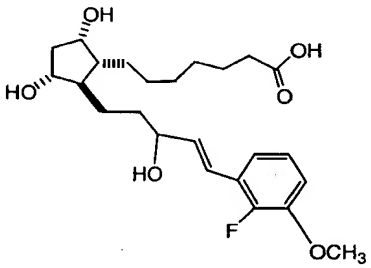
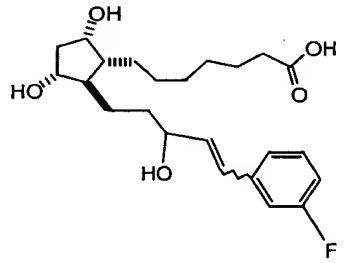
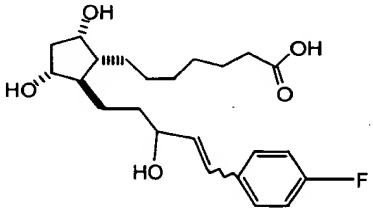
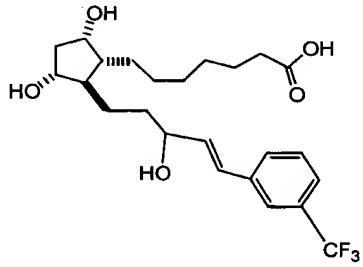


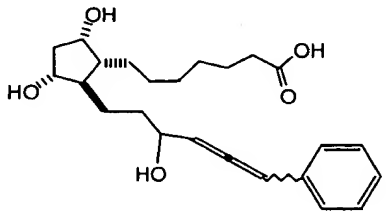
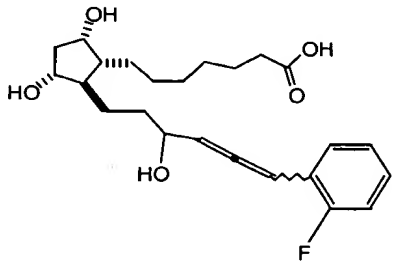
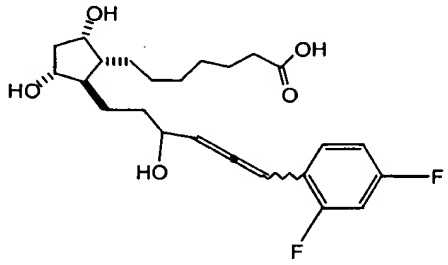
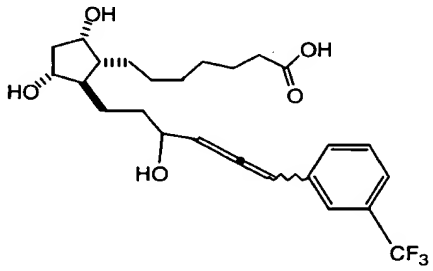
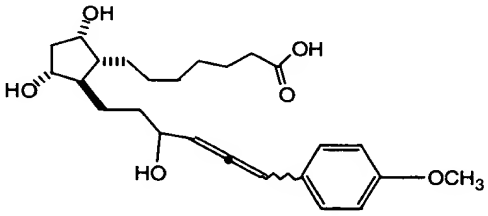
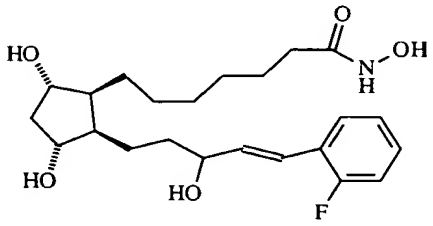
which are suitable for component A) are shown below in Tables 1 and 2.

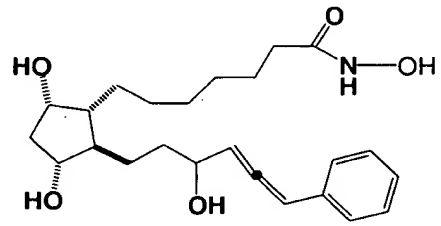
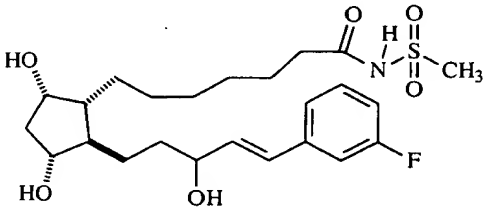
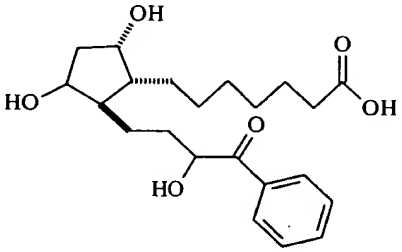
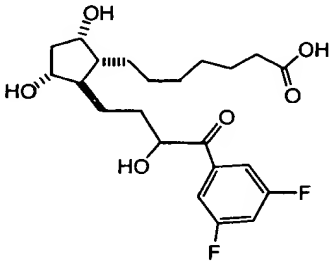
Table 1: Examples of Suitable PGF's for Component A)

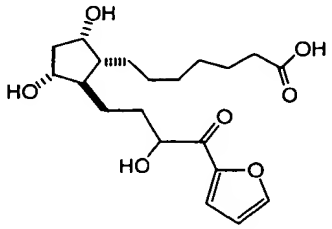
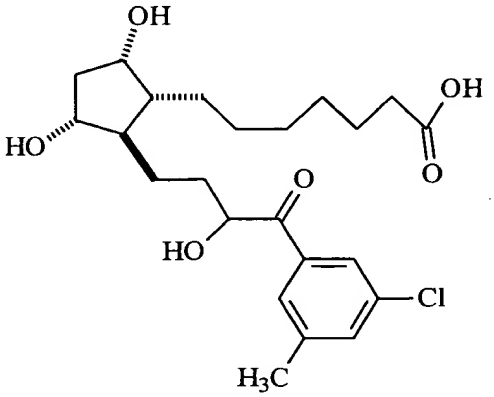
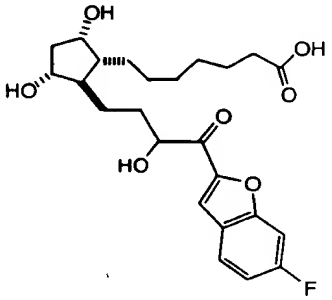
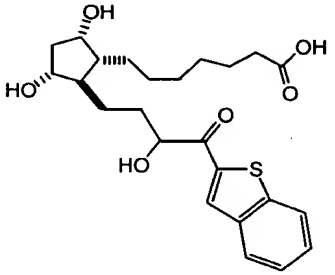
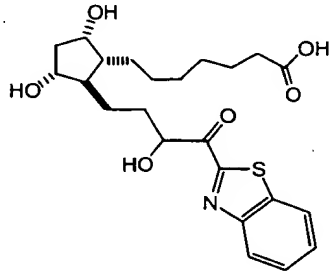
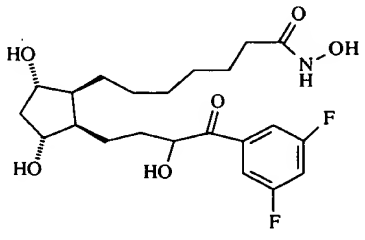
13,14-dihydro-16,17-Z-didehyro-17-(2-fluorophenyl)-17-trinor $PGF_{1\alpha}$	13,14-dihydro-16,17-*E*-didehyro-17-(2-fluorophenyl)-17-trinor $PGF_{1\alpha}$
	

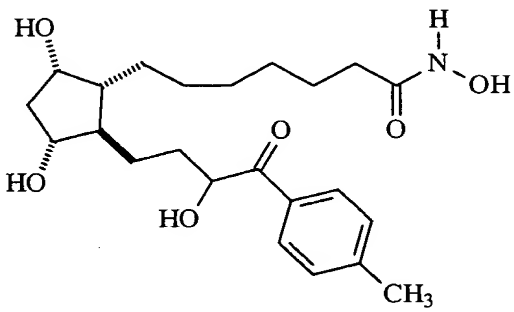
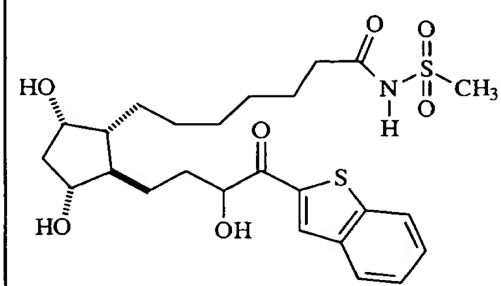
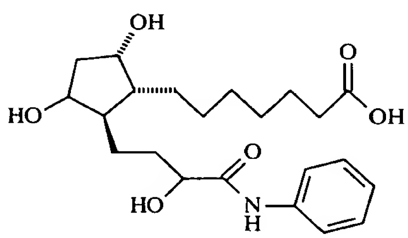
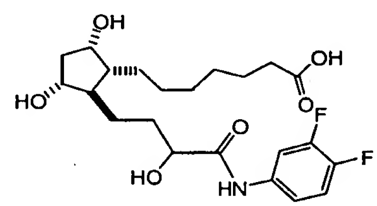
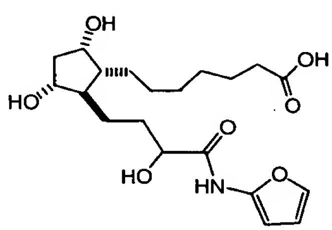
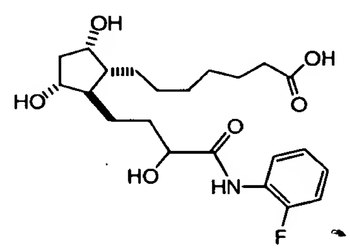
13,14-dihydro-*E*-16,17-didehyro-17-phenyl-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-*E*-16,17-didehyro-17-(2,4-dichlorophenyl)-17-trinor $PGF_{1\alpha}$ 
13,14-dihydro-*E*-16,17-didehyro-17-(2-fluoro-4-methylphenyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-*E*-16,17-didehyro-17-(2-fluoro-5-chlorophenyl)-17-trinor $PGF_{1\alpha}$ 

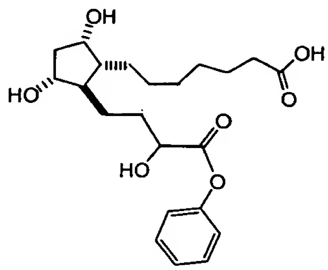
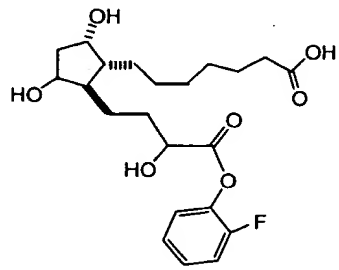
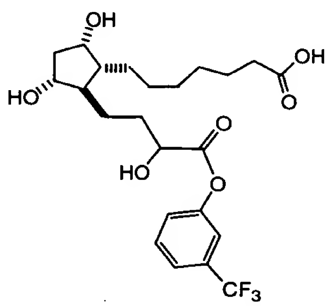
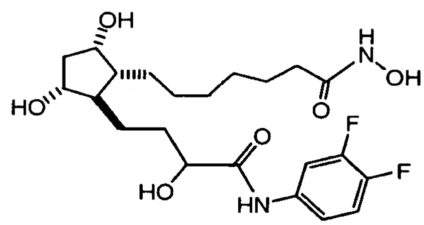
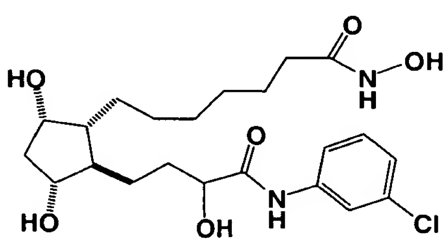
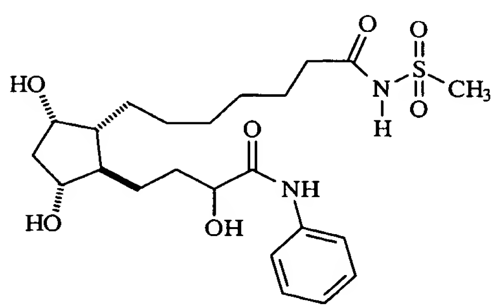
13,14-dihydro-*E*-16,17-didehyro-17-(2,5-difluorophenyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-*E*-16,17-didehyro-17-(2-fluoro-3-chlorophenyl)-17-trinor $PGF_{1\alpha}$ 
13,14-dihydro-*E*-16,17-didehyro-17-(2-fluoro-3-methoxyphenyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-16,17-didehyro-17-(3-fluorophenyl)-17-trinor $PGF_{1\alpha}$ 
13,14-dihydro-16,17-didehyro-17-(4-fluorophenyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-*E*-16,17-didehyro-17-(3-trifluoromethylphenyl)-17-trinor $PGF_{1\alpha}$ 

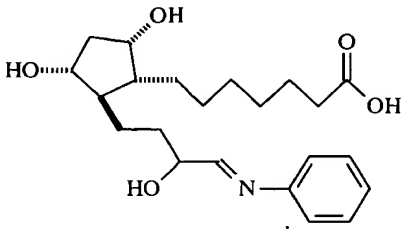
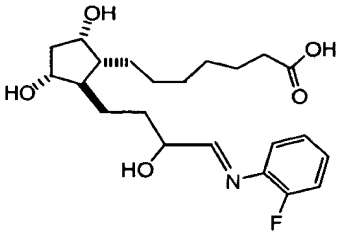
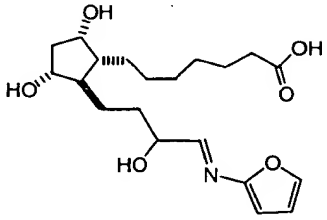
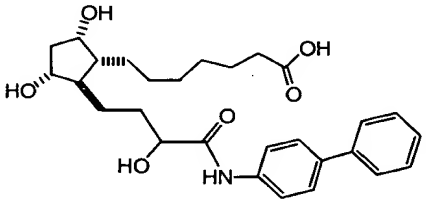
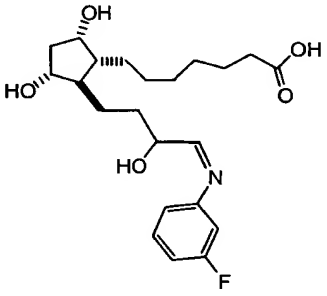
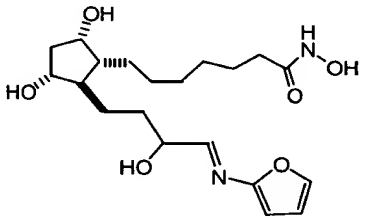
13,14-dihydro-16,17,17,18-dienyl-18-phenyl-18-dinor $PGF_{1\alpha}$ 	13,14-dihydro-16,17,17,18-dienyl-18-(2-fluorophenyl)-18-dinor $PGF_{1\alpha}$ 
13,14-dihydro-16,17,17,18-dienyl-18-(2,4-difluorophenyl)-18-dinor $PGF_{1\alpha}$ 	13,14-dihydro-16,17,17,18-dienyl-18-(3-trifluoromethylphenyl)-18-dinor $PGF_{1\alpha}$ 
13,14-dihydro-16,17,17,18-dienyl-18-(4-methoxyphenyl)-18-dinor $PGF_{1\alpha}$ 	13,14-dihydro-16,17-didehydro-17-(2-fluorophenyl)-17-trinor $PGF_{1\alpha}$ 1-hydroxamic acid 

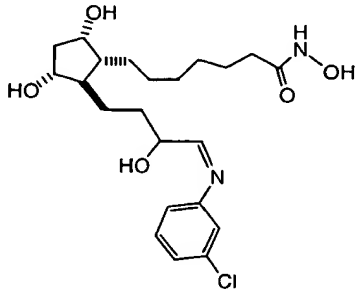
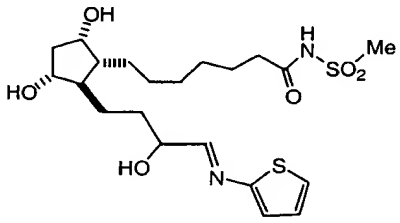
13,14-dihydro-16,17,17,18-dienyl-18-phenyl-18-dinorPGF$_{2\alpha}$ 1-hydroxamic acid 	13,14-dihydro-16,17-didehydro-17-3-fluorophenyl-17-trinor PGF$_{1\alpha}$ 1-N-methanesulfonamide 
13,14-dihydro-16-oxo-16-phenyl-16-tetranorPGF$_{1\alpha}$ 	13,14-dihydro-16-oxo-16-(3,5-difluorophenyl)-16-tetranor PGF$_{1\alpha}$ 

13,14-dihydro-16-oxo-16-(2-furanyl)-16-tetranor $PGF_{1\alpha}$ 	13,14-dihydro-16-oxo-16-(3-chloro-5-methylphenyl)-16-tetranor $PGF_{1\alpha}$ 
13,14-dihydro-16- oxo-16-(6-fluorobenzo-[*b*]-furanyl-16-(tetranor $PGF_{1\alpha}$ 	13,14-dihydro-16-oxo-16-(2-benzo[*b*]thienyl)-16-tetranor $PGF_{1\alpha}$ 
13,14-dihydro-16-oxo16-(2-benzothiazolyl)-16-tetranor $PGF_{1\alpha}$ 	13,14-dihydro-16-oxo-16-(3,5-difluorophenyl)-16-tetranor $PGF_{1\alpha}$ 1-hydroxamic acid 

13,14-dihydro-16-oxo-16-(4-methylphenyl)-16-tetranor PGF$_{1\alpha}$ 1-hydroxamic acid 	13,14-dihydro--16-oxo-16-(2-benzo[*b*]thienyl)-16-tetranor PGF$_{1\alpha}$ 1-N-methanesulfonamide 
13,14-dihydro-16-oxo-17-aza-17-phenyl-17-trinor PGF$_{1\alpha}$ 	13,14-dihydro-16-oxo-17-aza-17-(3,4-difluorophenyl)-17-trinor PGF$_{1\alpha}$ 
13,14-dihydro-16-oxo-17-aza-17-(2-furyl)-17-trinor PGF$_{1\alpha}$ 	13,14-dihydro-16-oxo-17-aza-17-(2-fluorophenyl)-17-trinor PGF$_{1\alpha}$ 

13,14-dihydro-16-oxo-16-phenoxy-16-tetranor $PGF_{1\alpha}$ 	13,14-dihydro-16-oxo-16-(2-fluorophenoxy)-16-tetranor $PGF_{1\alpha}$ 
13,14-dihydro-16-oxo-16-(3-trifluoromethylphenoxy)-16-tetranor $PGF_{1\alpha}$ 	13,14-dihydro-16-oxo-17-aza-17-(3,4-difluorophenyl)-17-trinor $PGF_{1\alpha}$ 1-hydroxamic acid 
13,14-dihydro-16-oxo-17-amino-17-(3-chlorophenyl)-17-trinor $PGF_{1\alpha}$ 1-hydroxamic acid: 	13,14-dihydro-16-oxo-17-amino-17-phenyl-17-trinor $PGF_{1\alpha}$ 1-methane sulfonamide 

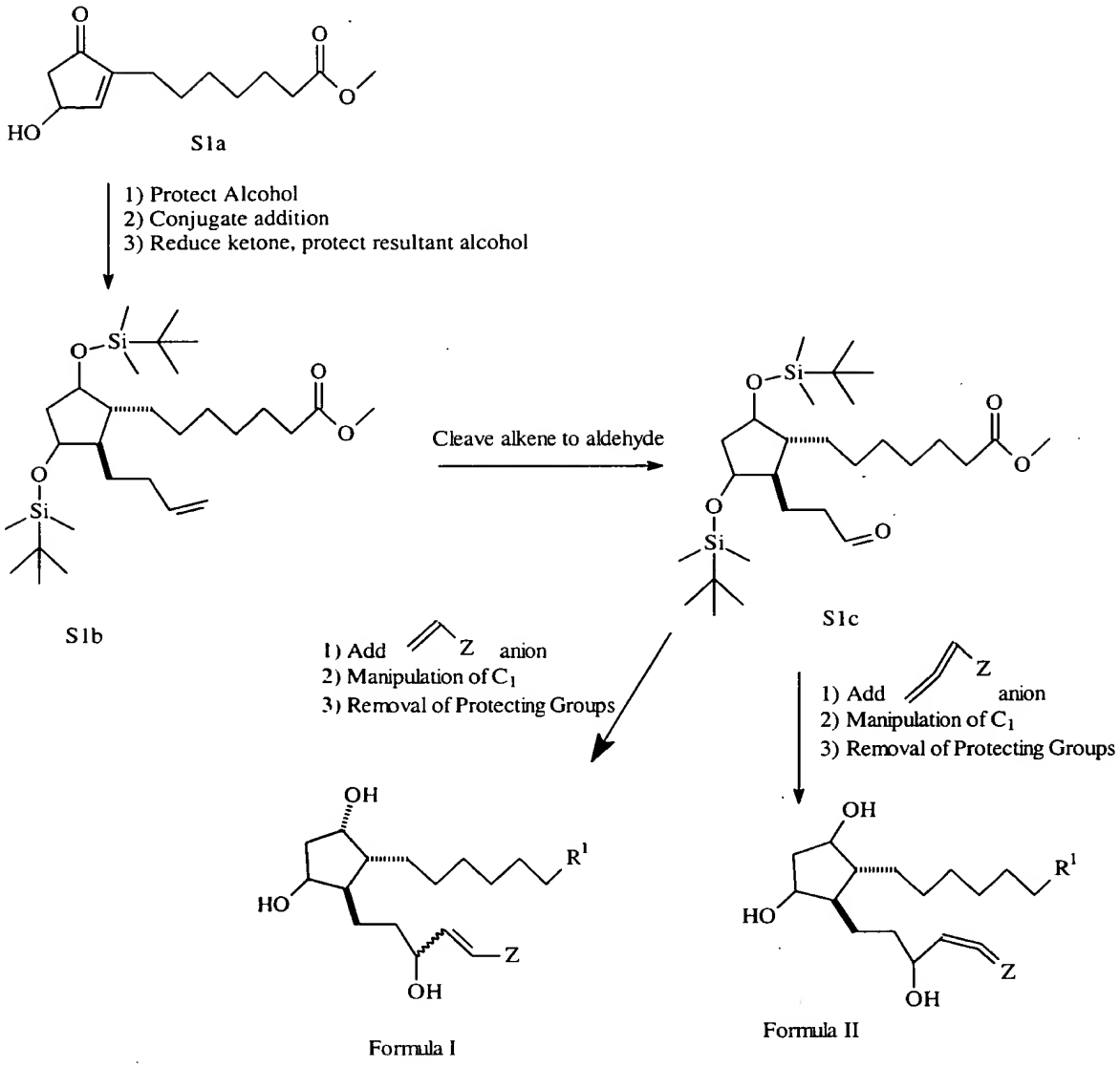
13,14-dihydro-16,17-didehydro-17-aza-17-phenyl-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-16,17-didehydro-17-aza-17-(2-fluorophenyl)-17-trinor $PGF_{1\alpha}$ 
13,14-dihydro-16,17-didehydro-17-aza-17-(2-furanyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-16-oxo-17-aza--17-(4-phenylphenyl)-17-trinor $PGF_{1\alpha}$ 
13,14-dihydro-16,17-didehydro-17-aza-17-(3-fluorophenyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-16,17-didehydro-17-aza-17-(2-furanyl)-17- trinor $PGF_{1\alpha}$ 1-hydroxamic acid 

13,14-dihydro-16,17-didehydro-17-aza-17-(3-chlorophenyl)-17-trinor $PGF_{1\alpha}$ 1-hydroxamic acid	13,14-dihydro-16,17-didehydro-17-aza-17-(2-thienyl)-17-trinor $PGF_{1\alpha}$ 1-methanesulfonamide
	

Where Me in the table above represents a methyl group.

The PGF's in Table 1 can be prepared using conventional organic syntheses. Preferred syntheses are carried out using reaction schemes 1, 2, and 3. **Scheme 1** describes a general reaction scheme for making PGFs wherein X is -CH=CH- (**Formula I**) or -CH=C=CH- (**Formula II**). **Scheme 2** describes a general reaction scheme for making PGFs wherein X is -C(O)- (**Formula III**) or -C(O)Y- (**Formula IV**). **Scheme 3** describes a general reaction scheme for making PGFs wherein X is -CH=N- (**Formula V**).

Scheme 1



In **Scheme 1**, R^1 and Z are as defined above. The methyl 7(3-(R)-hydroxy-5-oxo-1-cyclopent-1-yl) heptanoate (**S1a**) depicted as starting material for **Scheme 1** is commercially available (such as from Sumitomo Chemical or Cayman Chemical).

In **Scheme 1**, methyl 7-(3-(R)-hydroxy-5-oxo-1-cyclopent-1-yl) heptanoate (**S1a**) is reacted with a silylating agent and base in a solvent that will allow the silylation to proceed. Preferred silylating agents include tert-butyldimethylsilyl chloride and tert-butyldimethylsilyl trifluoromethanesulphonate. The most preferred silylating agent is tert-

butyldimethylsilyl trifluoromethanesulphonate. Preferred bases include triethylamine, trimethylamine, and 2,6-lutidine. More preferred bases include triethylamine and 2,6-lutidine. The most preferred base is 2,6-lutidine. Preferred solvents include halogenated hydrocarbon solvents with dichloromethane being the most preferred solvent. The reaction is allowed to proceed at a temperature of preferably -100°C to 100°C, more preferably -80°C to 80°C, and most preferably -70°C to 23°C.

The resulting silylated compound is isolated by methods known to one of ordinary skill in the art. Such methods include extraction, solvent evaporation, distillation, and crystallization. Preferably, the silyl ether is purified after isolation by distillation under vacuum.

The silylated compound is then reacted with the cuprate generated *via* Grignard formation of the appropriate alkenyl bromide as disclosed, for example, in the following references: H.O. House et. al., "The Chemistry of Carbanions: A Convenient Precursor for the Generation of Lithium Organocuprates", J. Org. Chem. Vol. 40 (1975) pp. 1460-69 ; and P. Knochel et. al., "Zinc and Copper Carbenoids as Efficient and Selective a'/d' Multicoupling Reagents", J. Amer. Chem. Soc. Vol. 111 (1989) p. 6474-76. Preferred alkenyl bromides include 4-bromo-1-butene, 4-bromo-1-butyne, 4-bromo-2-methyl-1-butene, and 4-bromo-2-ethyl-1-butene. The most preferred alkenyl bromide is 4-bromo-1-butene. Preferred solvents include ethereal solvents, of which diethyl ether and tetrahydrofuran are preferred. The most preferred solvent is tetrahydrofuran. The Grignard reagent is allowed to form at a temperature of 100°C to 23°C, more preferably 85°C to 30°C, and most preferably 75°C to 65°C. The reaction time is preferably 1 to 6 hours, more preferably 2 to 5 hours, and most preferably 3 to 4 hours.

Once the Grignard reagent is formed, the cuprate is generated from the alkenyl magnesium species. The temperature range for cuprate formation is -100°C to 0°C. The preferred temperature range is -80°C to -20°C. The more preferred temperature range is -75°C to -50°C. The preferred reaction time is 30 minutes to 6 hours, more preferably 45 minutes to 3 hours. The most preferred reaction time is 1 to 1.5 hours.

The alkene thus formed is isolated by methods known to one of ordinary skill in the art. Such methods include extraction, solvent evaporation, distillation, and

crystallization. Preferably, the alkene is purified by flash chromatography on silica gel (Merck, 230-400 mesh) using 10% EtOAc/hexanes as the eluent. (EtOAc represents ethyl acetate.)

The alkene is then reacted with a hydride reducing agent and a polar, protic solvent to give the C-9 alcohol. Preferred reducing agents include lithium aluminum hydride, sodium borohydride, and L-selectride. More preferred reducing agents include sodium borohydride, and L-selectride. The most preferred reducing agent is sodium borohydride. Preferred solvents include methanol, ethanol, and butanol. The most preferred solvent is methanol. The reduction is carried out at a temperature of -100°C to 23°C. The preferred temperature range is -60°C to 0°C. The most preferred temperature range is -45°C to -20°C.

The resulting alcohol is isolated by methods known to one of ordinary skill in the art. Such methods include extraction, solvent evaporation, distillation, and crystallization. Preferably, the alcohol is purified by flash chromatography on silica gel (Merck, 230-400 mesh) using 20% EtOAc/hexanes as the eluent.

The resultant alcohol can be protected as described previously herein. Preferred silylating agents in this case also include tert-butyldimethylsilyl chloride and tert-butyldimethylsilyl trifluoromethanesulfonate. The most preferred silylating agent is tert-butyldimethylsilyl trifluoromethanesulfonate. Preferred bases include triethylamine, trimethylamine, and 2,6-lutidine. More preferred bases include triethylamine and 2,6-lutidine. The most preferred base is 2,6-lutidine. Preferred solvents include halogenated hydrocarbon solvents with dichloromethane being the most preferred solvent. The reaction is allowed to proceed at a temperature of preferably -100°C to 100°C, more preferably -80°C to 80°C, and most preferably -70°C to 23°C.

The resulting silylated compound, **S1b** is isolated by methods known to one of ordinary skill in the art. Such methods include extraction, solvent evaporation, distillation, and crystallization. Preferably, the silyl ether is purified after isolation by distillation under vacuum, giving compound **S1b**.

The protected alcohol is then treated with a form of osmium and sodium periodate in a solvent in which both are soluble. Preferred forms of osmium include osmium

tetraoxide and potassium osmate. Preferred solvent systems include 1:1 mixtures of acetic acid and water and 1:1:2 mixtures of water, acetic acid and THF. (THF represents tetrahydrofuran.) The result of this treatment is the aldehyde, **S1c**.

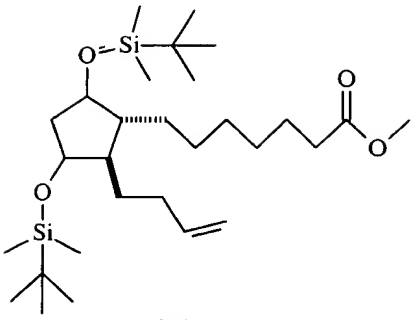
The compound **S1c** is isolated by methods known to one of ordinary skill in the art. Such methods include extraction, solvent evaporation, distillation, and crystallization. Preferably, **S1c** is purified by flash chromatography on silica gel (Merck, 230-400 mesh) using 20% EtOAc/hexanes as the eluent.

The key intermediate aldehyde depicted as **S1c** can be reacted with a variety of unsaturated alkenyl anion nucleophiles to provide the C-9 and C-11-protected 13,14-dihydro-prostaglandin $F_{1\alpha}$ derivatives.

The resulting compounds can be isolated, but are generally deprotected using techniques known to one of ordinary skill in the art, and optionally, manipulated at C-1 to provide the desired acid derivative at R^1. For example, the condensation of a methyl ester with an amine or a hydroxylamine provides an amide or a hydroxamic acid compound, respectively. After any such manipulation at C-1, the compounds are isolated as the final 13,14-dihydro-15-substituted-15-pentanor prostaglandin $F_{1\alpha}$ derivative, **Formula I**.

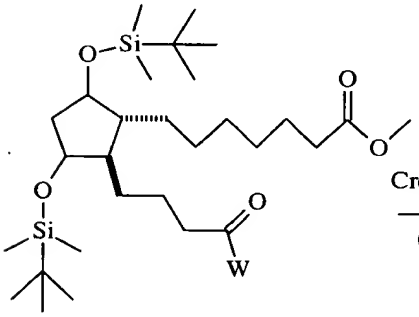
Compounds depicted by **Formula II** can be made directly from intermediate **S1c** in a manner similar to that for compounds depicted by **Formula I** substituting the appropriate allene anion. With allene nucleophiles, the reaction is carried out preferably at -80°C to 0°C, more preferably -80°C to -20°C, and most preferably -80°C to -40°C. Preferred bases for the reaction include *n*-butyl lithium, *s*-butyl lithium, and *t*-butyl lithium. The most preferred base is *n*-butyl lithium. Preferred solvents for the reaction are ether solvents. Preferred solvents include diethyl ether, and tetrahydrofuran. The most preferred solvent is tetrahydrofuran. With heterocyclic nucleophiles, preferred solvents include ethereal solvents. More preferred ethereal solvents include diethyl ether, dibutyl ether and tetrahydrofuran. The most preferred ethereal solvent is tetrahydrofuran. After isolation, similar C-1 manipulations and/or deprotection of the functional groups ensues using techniques known to one of ordinary skill in the art.

Scheme 2



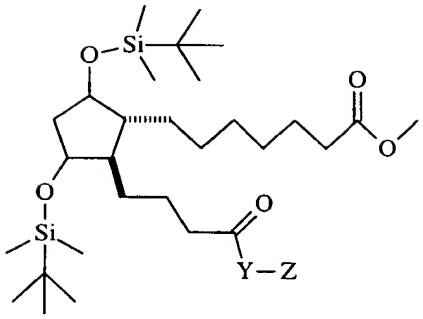
S1b

1) Add borane to alkene
2) Oxidize to aldehyde or acid



S2a W = H or OH

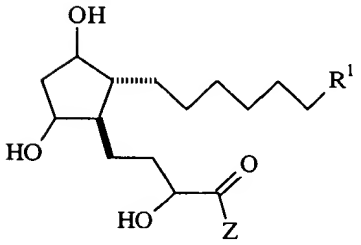
Create Ester/ Amide
(if W = OH)



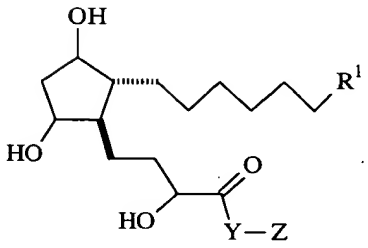
S2b

1) Add Z- anion (if W=H)
2) Oxidize to ketone
3) Remove ester
4) Oxidize ketone enolate to alpha hydroxy ketone
5) Remove protecting groups on the alcohols/ Optionally manipulate C1 if other than acid is desired

1) Selective Removal of Methyl Ester
2) Oxidize alpha to ester via ester enolat
3) Remove protecting groups on alcohol Optionally manipulate C1 if other tha Acid is desired



Formula III



Formula IV

In **Scheme 2**, R^1, Y, and Z are as defined above. The protected alcohol **S1b** (from **Scheme 1**) is treated with a hydroborating reagent in an ethereal solvent, followed by oxidative removal of the boron reagent with a suitable oxidant to give a compound of the type **S2a**. Preferred hydroborating reagents include monochloroborane-dimethylsulfide, diborane, borane-tetrahydrofuran and borane-dimethylsulfide. The most preferred hydroborating reagent is borane-dimethylsulfide. Preferred ethereal solvents include THF and diethyl ether. The most preferred solvent is THF. The reaction is carried out from about 1 to about 24 hours at a temperature of about -20 °C to about +30 °C. The preferred temperature range is about 0 °C to about +20 °C. The hydroborated product of this reaction may then be oxidatively removed to the alcohol using alkaline hydrogen peroxide (See *Boranes in Organic Chemistry*, H. C. Brown, Cornell University Press, Ithaca, NY 1972, pp. 321-325), which may then be oxidized to either the aldehyde (W= H) or to the acid (W= OH) using methods known to one of ordinary skill in the art. Alternatively, the hydroborated product may be directly oxidized to the aldehyde or acid by treatment with chromic acid or a Cr(VI) salt. Such salts include pyridinium chlorochromate (PCC) and dichlorochromate. See Brown, H. C.; Kulkarni, Rao, and Patil, Tetrahedron, 1986, 45515. The preferred method is treatment of the hydroborated product with PCC in dichloromethane at room temperature. The result of these manipulations is a compound of the type **S2a**.

The compound **S2a** is isolated by methods known to one of ordinary skill in the art. Such methods include extraction, solvent evaporation, distillation, and crystallization. Preferably, **S2a** is purified by flash chromatography on silica gel (Merck, 230-400 mesh) using 20% EtOAc/hexanes as the eluent with 0.1% acetic acid added if W = OH.

The key intermediate aldehyde depicted as **S2a** can be reacted with a variety unsaturated carbon nucleophiles to provide the C-9 and C-11-protected 13,14-dihydro-16-tetranor prostaglandin $F_{1\alpha}$ derivatives of **Formula III**.

With aromatic and heteroaromatic nucleophiles, the reaction is carried out preferably at -80°C to 0°C, more preferably -80°C to -20°C, and most preferably -80°C to -40°C. Preferred bases for the reaction include *n*-butyl lithium, *s*-butyl lithium, lithium diisopropylamide, and *t*-butyl lithium. The most preferred base is *n*-butyl lithium.

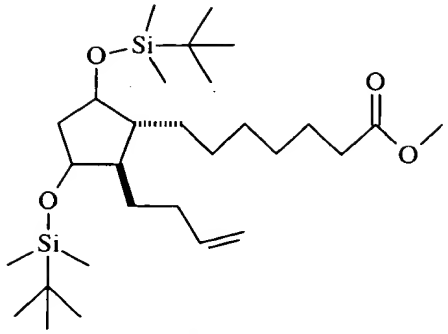
Preferred solvents for the reaction are ether solvents. Preferred solvents include diethyl ether, and tetrahydrofuran. The most preferred solvent is tetrahydrofuran. With heterocyclic nucleophiles, preferred solvents include ethereal solvents. More preferred ethereal solvents include diethyl ether, dibutyl ether and tetrahydrofuran. The most preferred ethereal solvent is tetrahydrofuran.

The resulting alcohol can be isolated, but is generally oxidized as a crude isolate. The oxidation of benzylic alcohols to benzylic ketones is well known in the art. The preferred reagents to effect this reaction include $KMnO_4$, MnO_2, chromic acid, Jones' reagent, Collins' reagent, and PCC. The most preferred method is oxidation at room temperature in dichloromethane with PCC for about 4 hours. The ketones are isolated by column chromatography using 20% hexanes/ethyl acetate as solvent. The ester is then removed using standard conditions. See Greene and Wuts, Protecting Groups in Organic Synthesis, Wiley Interscience, NY pp. 224-276. The free acid is then treated with 2.1 equivalents of a strong nitrogen base to effect deprotonation both of the acid and adjacent to the benzylic ketone. Such bases include LDA. This enolate is reacted with a peroxidizing agent which has the effect of oxidizing the compound to deliver the alpha-hydroxy ketone. Such reagents include *meta* -chloroperoxybenzoic acid, dimethyl dioxirane, Davis' reagent and peracetic acid. The crude product may be isolated or the remaining protecting groups may be removed. At this point manipulation of the acid at C-1 may take place. For example, re-esterifying, making the amide, the hydroxamic acid or the sulfonamide using methods known to one of ordinary skill in the art may be performed to yield compounds according to **Formula III**.

Compounds depicted by **Formula IV** can be made from intermediate **S2b**. In this case, condensation of the free acid is readily achieved with a variety of alcohols and amines, either by the use of coupling agents such as dicyclohexylcarbodiimide ("DCC"), or by activating the acid with, for example, oxalyl chloride. Following this is the selective removal of the methyl esters as described in Greene and Wuts, Protecting Groups in Organic Synthesis, Wiley Interscience, NY pp. 224-276, and the oxidation of the ester enolates using the same technique described above for the ketone intermediates. Similarly,

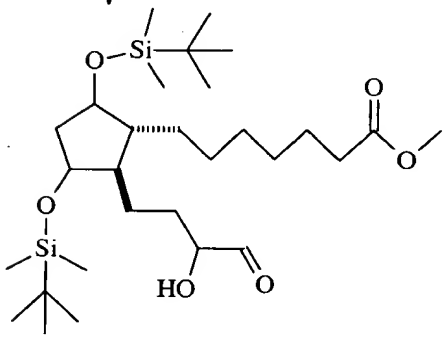
as described above, the remaining protecting groups are removed and the desired manipulation of C-1 is effected, yielding compounds of **Formula IV.**

Scheme 3



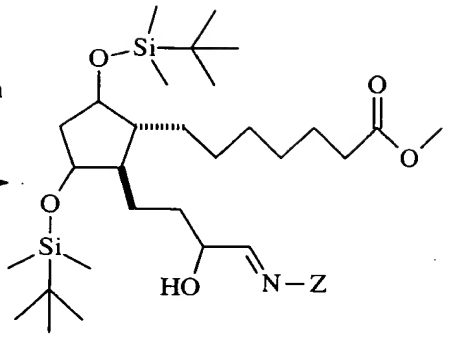
S1b

1) Oxidize alkene to diol
2) Optionally selectively protect secondary alcohol
3) Selectively oxidize primary alcohol



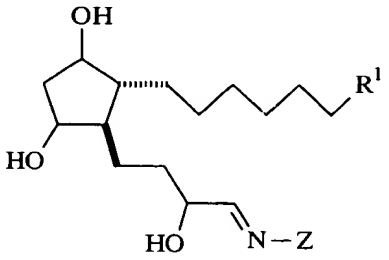
S3a Q = protecting group or H

Condensation with appropriate amine (NH_zZ)



S3b

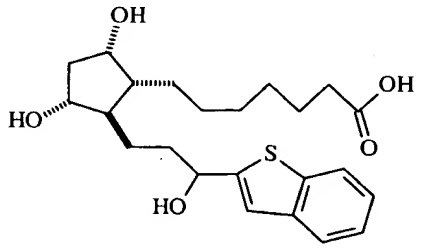
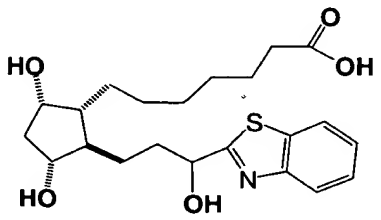
Remove protecting groups on alcohols/ manipulate C1

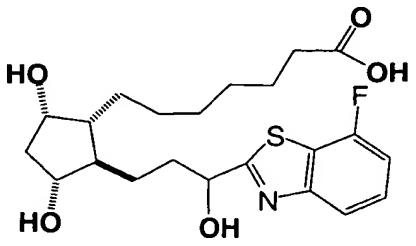
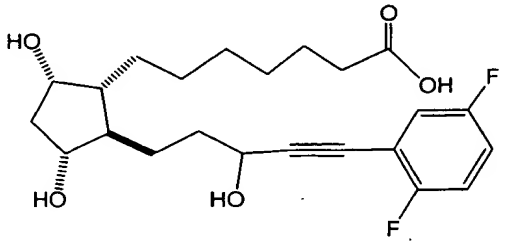
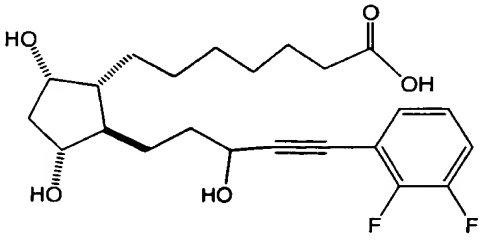
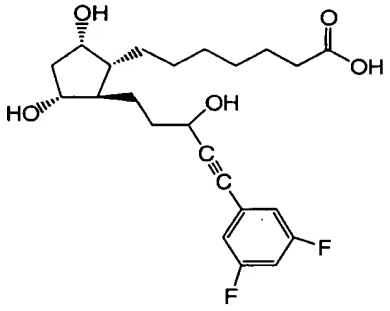
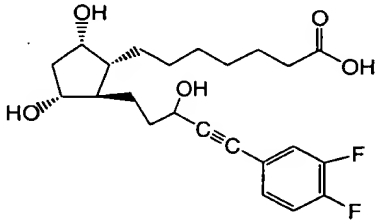
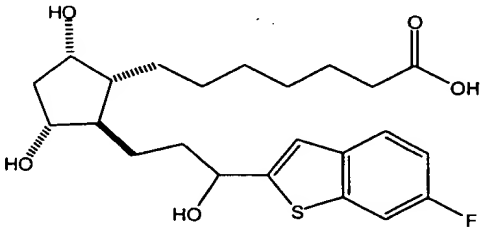


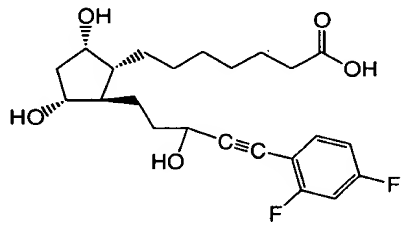
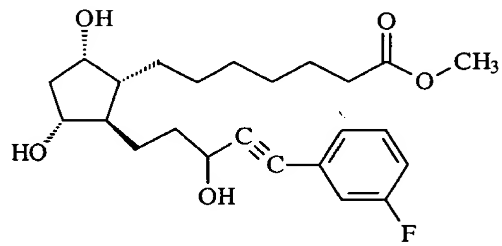
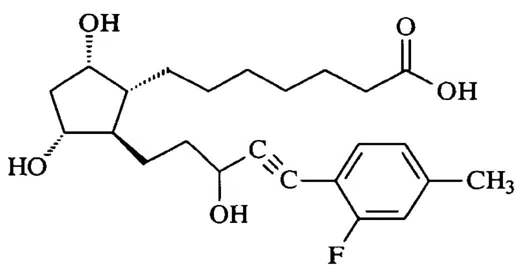
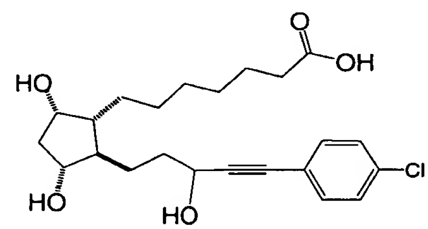
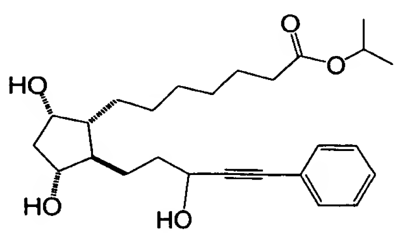
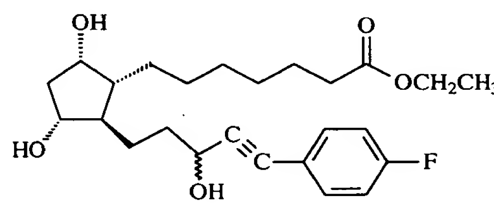
Formula V

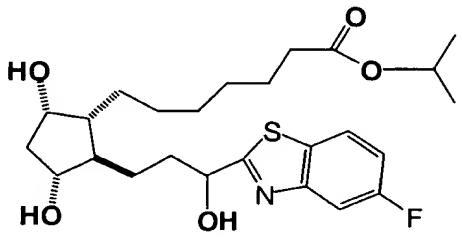
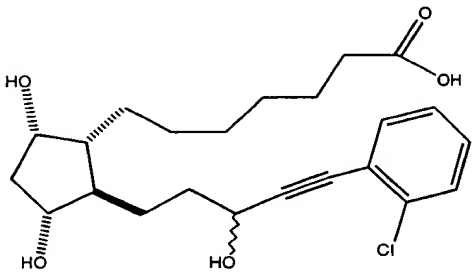
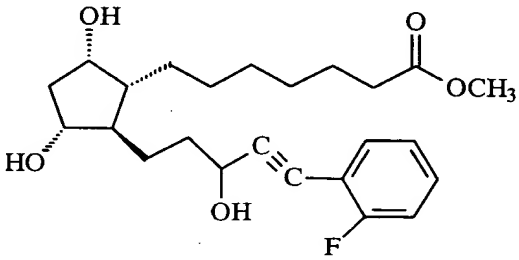
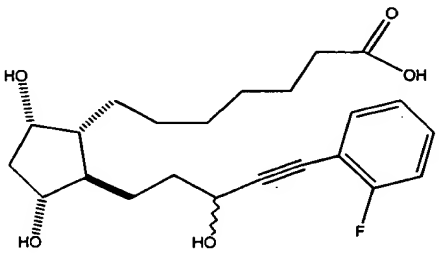
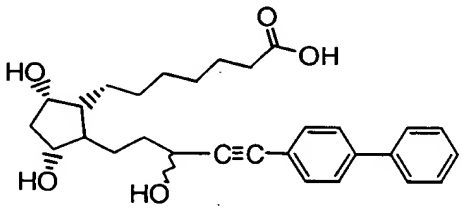
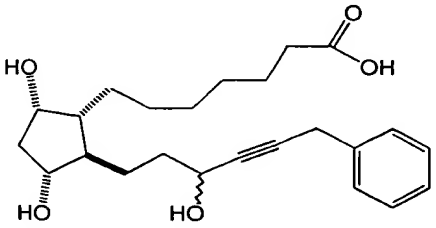
In **Scheme 3**, R^1 and Z are as defined above. The alkene **S1b** (from **Scheme 1**) is treated with an osmium salt and with an optional catalyst reoxidant, preferably *N*-methyl morpholine *N*-oxide ("NMO"), to give the diol. This diol is isolated by extraction and purified by silica gel chromatography. The diol is then oxidized selectively to the *alpha* hydroxy aldehyde. This may be accomplished in several ways. For example, a selective oxidant such as DMSO-oxalyl chloride may be used. ("DMSO" represents dimethylsulfoxide.) Alternatively, the primary alcohol may be selectively protected, then the secondary alcohol protected, then the protection on the primary alcohol may then be removed and the alcohol oxidized as described above in **Scheme II**. However, the preferred method is the addition of a *o*-bromo-benzyl bromide protecting group, which can be removed with concomitant oxidation by tributyl tin hydride and like reagents. This technique yields compounds of the type **S3a**, wherein Q = H. From this step follows the condensation of the aldehyde with an amine to form an imine of the type **S3b**. Appropriate removal of protecting groups and manipulation of C-1 as stated above in **Schemes I** and **II** yields compounds of **Formula V**.

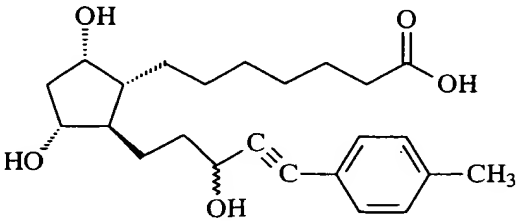
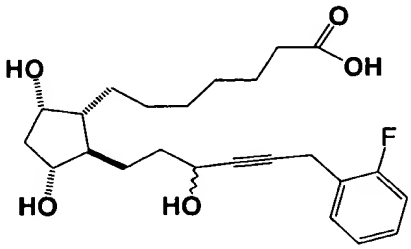
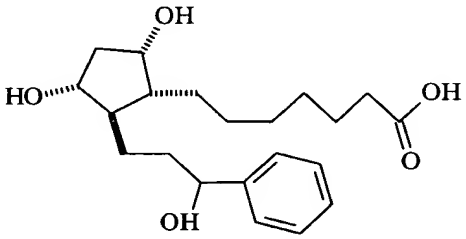
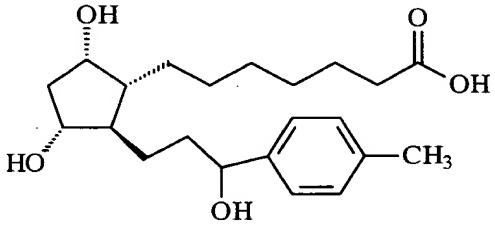
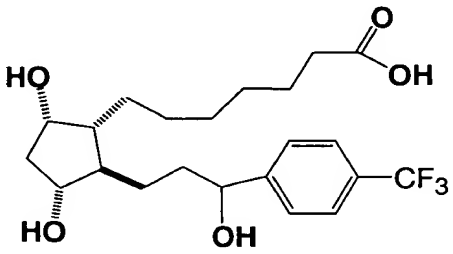
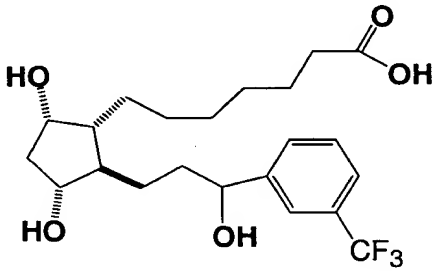
Table 2: Examples of Suitable PGF's

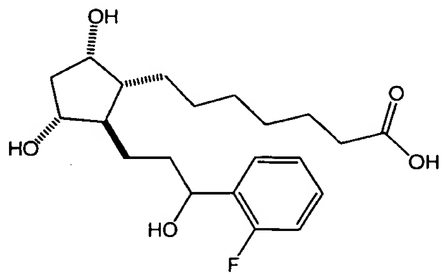
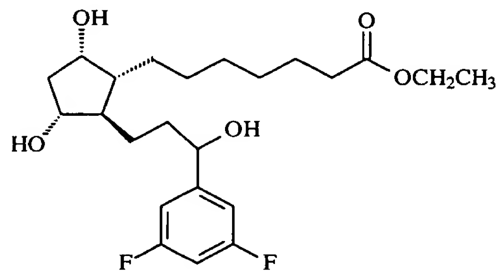
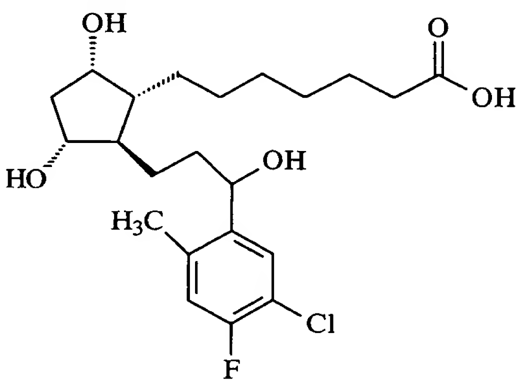
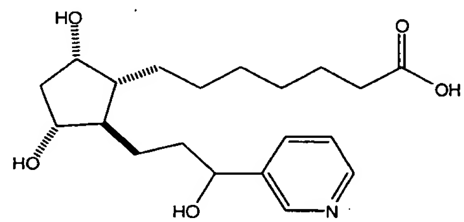
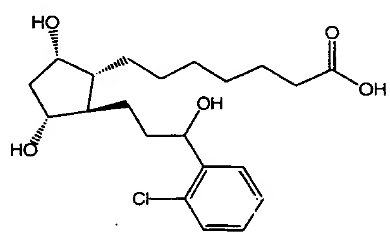
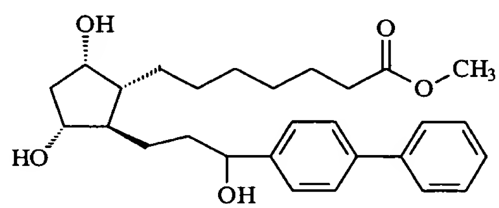
13,14-dihydro-15-(2-benzothienyl)-15-pentanor $PGF_{1\alpha}$	13,14-dihydro-15-(2-benzothiazolyl)-15-pentanor $PGF_{1\alpha}$
	

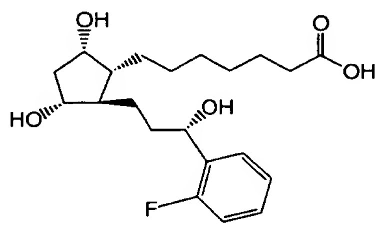
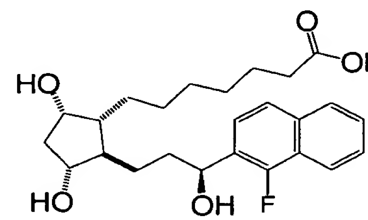
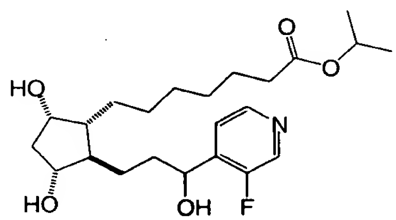
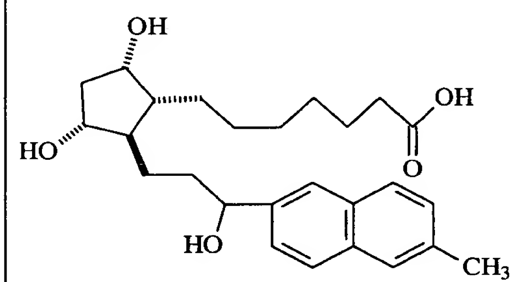
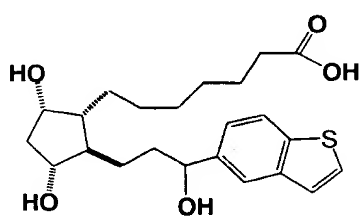
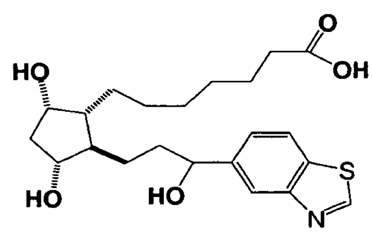
13,14-dihydro-15-(8-fluoro-2-benzothiazolyl)-15- pentanor $PGF_{1\alpha}$ 	13,14-dihydro-16,17-ynyl17-(2,5-difluorophenyl)-17-trinor $PGF_{1\alpha}$ 
13,14-dihydro-16,17-ynyl-17-(2,3-difluorophenyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-16,17-ynyl-17-(3,5-difluorophenyl)-17-trinor $PGF_{1\alpha}$ 
13,14-dihydro-16,17-ynyl-17-(3,4-difluorophenyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-15-(6-fluoro-2-benzothienyl)-15-pentanor $PGF_{1\alpha}$ 

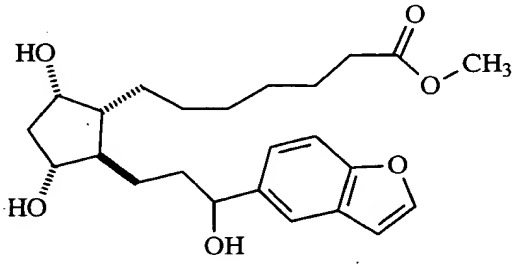
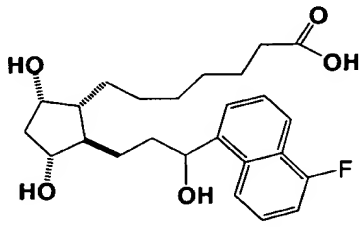
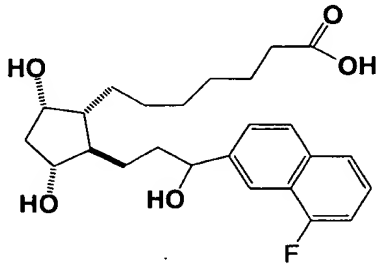
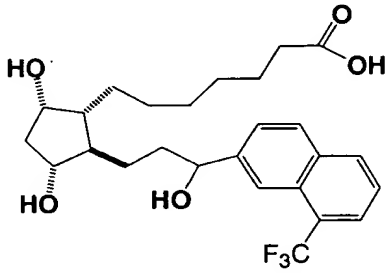
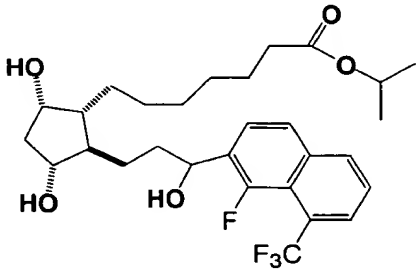
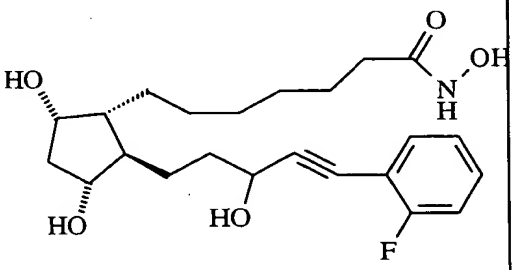
3,14-dihydro-16,17-ynyl-17-(2,4-difluorophenyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-16,17-ynyl-17-(3-fluorophenyl)-17-trinor $PGF_{1\alpha}$ methyl ester 
13,14-dihydro-16,17-ynyl-17-(2-fluoro-4-methylphenyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-16,17-ynyl-17-(4-chlorophenyl)-17-trinor $PGF_{1\alpha}$ 
13,14-dihydro-16,17-ynyl-17-phenyl-17-trinor $PGF_{1\alpha}$ isopropyl ester 	13,14-dihydro-16,17-ynyl-17-(4-fluorophenyl)-17-trinor $PGF_{1\alpha}$ ethyl ester 

13,14-dihydro-15-(5-fluoro-2-benzothiazolyl)-15-pentanor $PGF_{1\alpha}$ isopropyl ester 	13,14-dihydro-16,17-ynyl-17-(2-chlorophenyl)-17-trinor $PGF_{1\alpha}$ 
13,14-dihydro-16,17-ynyl-17-(2-fluorophenyl)-17-trinor $PGF_{1\alpha}$ methyl ester 	13,14-dihydro-16,17-ynyl-17-(2-fluorophenyl)-17-trinor $PGF_{1\alpha}$ 
13,14-dihydro-16,17-ynyl-17-(4-phenyl-phenyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-16,17-ynyl-18-phenyl-18-dinor $PGF_{1\alpha}$ 

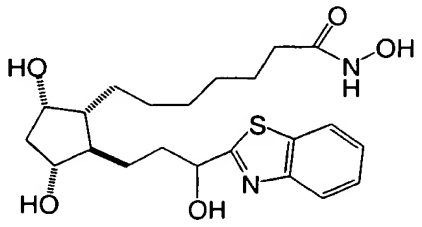
13,14-dihydro-16,17-ynyl-17-(4-methylphenyl)-17-trinor $PGF_{1\alpha}$ 	13,14-dihydro-16,17-ynyl-18-(2-fluorophenyl)-18-dinor $PGF_{1\alpha}$ 
13,14-dihydro-15-phenyl-15-pentanor $PGF_{1\alpha}$ 	13,14-dihydro-15-(4-methylphenyl)-15-pentanor $PGF_{1\alpha}$ 
13,14-dihydro-15-(4-trifluoromethylphenyl)-15-pentanor $PGF_{1\alpha}$ 	13,14-dihydro-15-(3-trifluoromethylphenyl)-15-pentanor $PGF_{1\alpha}$ 

13,14-dihydro-15-(2-fluorophenyl)-15-pentanor $PGF_{1\alpha}$ 	13,14-dihydro-15-(3,5 difluorophenyl)-15-pentanor $PGF_{1\alpha}$ ethyl ester 
13,14-dihydro-15-(3-chloro-4-fluoro-6-methylphenyl)-15pentanor $PGF_{1\alpha}$ 	13,14-dihydro-15-(3-pyridinyl)-15-pentanor $PGF_{1\alpha}$ 
13,14-dihydro-15-(2-chlorophenyl)-15-pentanor $PGF_{1\alpha}$ 	13,14-dihydro-15-(4-phenylphenyl)-15-pentanor $PGF_{1\alpha}$ methyl ester 

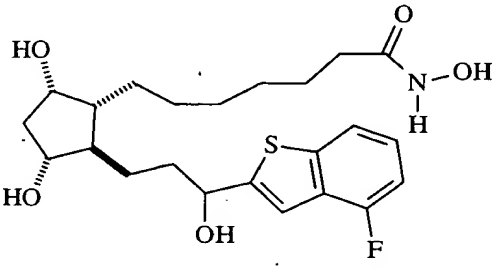
13,14-dihydro-15-*S*-(2-fluorophenyl)-15-pentanor $PGF_{1\alpha}$ 	13,14-dihydro-15-*S*-(2-fluoronaphthyl)-15-pentanor $PGF_{1\alpha}$ 
13,14-dihydro-15-(3-fluoro-4-pyridyl)-15-pentanor $PGF_{1\alpha}$ isopropyl ester 	13,14-dihydro-15-(6-methylnaphth-2-yl)-15-pentanor $PGF_{1\alpha}$ 
13,14-dihydro-15-(benzo(*b*)thiophen-5-yl)-15-pentanor $PGF_{1\alpha}$ 	13,14-dihydro-15-(6-benzothiazol-5-yl)-15-pentanor $PGF_{1\alpha}$ 

13,14-dihydro-15-(benzofuran-5-yl)-15-pentanor $PGF_{1\alpha}$ methyl ester 	13,14-dihydro-15-(5-fluoronaphth-1-yl)-15-pentanor $PGF_{1\alpha}$ 
13,14-dihydro-15-(8-fluoro-2-naphthyl)-15-pentanor $PGF_{1\alpha}$ 	13,14-dihydro-15-(8-trifluoromethyl-2-naphthyl)-15-pentanor $PGF_{1\alpha}$ 
13,14-dihydro-15-(1-fluoro-3-trifluoromethyl-2-naphthyl)-15-pentanor $PGF_{1\alpha}$ isopropyl ester 	13,14-dihydro-16,17-ynyl-17-(2-fluorophenyl)-17-trinor $PGF_{1\alpha}$ 1-hydroxamic acid 

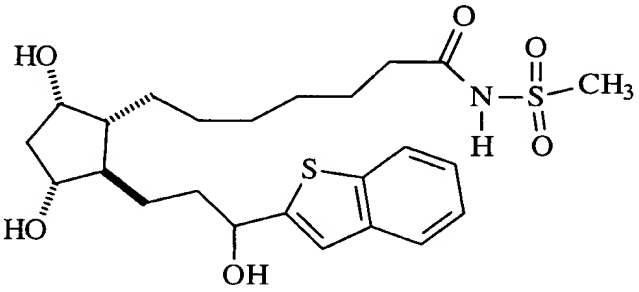
13,14-dihydro-15-(benzothiazolyl)-15-pentanor $PGF_{1\alpha}$ 1-hydroxamic acid



13,14-dihydro-15-(4-fluoro-2-benzothienyl)-15-pentanor $PGF_{1\alpha}$ 1-hydroxamic acid

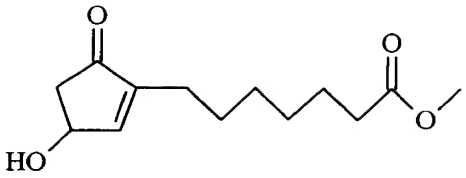


13,14-dihydro-15-(2-benzothienyl)-15-pentanor $PGF_{1\alpha}$ 1-N-methanesulfonamide



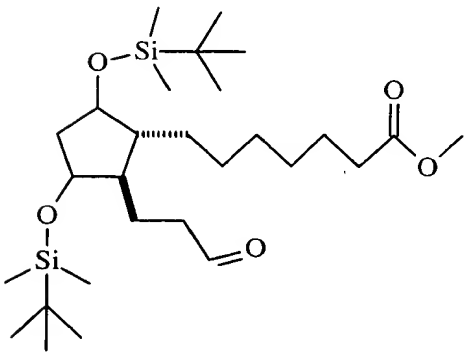
The PGF's in Table 2 can be prepared by conventional organic syntheses. A preferred synthesis is reaction scheme 4.

Scheme 4



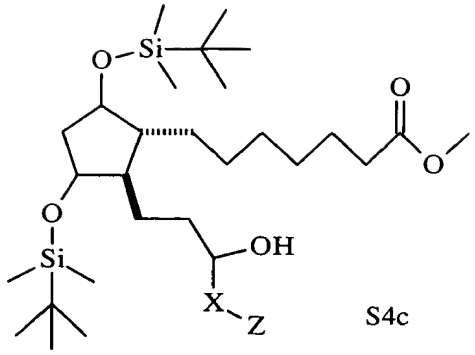
S4a

1) Protect alcohol
2) Conjugate addition
3) Reduce ketone, protect resultant alcohol
4) Cleave alkene to aldehyde



S4b

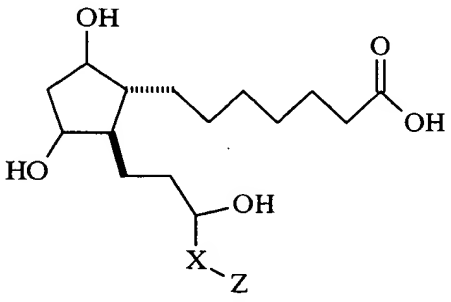
X^-Z anion



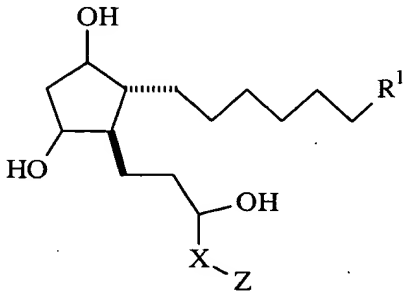
S4c

1) Removal of Protecting Groups

1) Manipulation of C1 ester
2) Removal of protecting grou



Formula VI



Formula VII

In **Scheme 4**, R^1, R^2, X, and Z are as defined above. The methyl 7(3-(R)-hydroxy-5-oxo-1-cyclopent-1-yl) heptanoate (**S4a**) depicted as starting material for **Scheme 4** is commercially available (such as from Sumitomo Chemical or Cayman Chemical).

The C_{11} alcohol of methyl 7-(3-(R)-hydroxy-5-oxo-1-cyclopent-1-yl) heptanoate (**S4a**) is protected with a suitable protecting group. The most preferred protecting group is a silyl group. In the above **Scheme 4**, methyl 7-(3-(R)-hydroxy-5-oxo-1-cyclopent-1-yl) heptanoate (**S4a**) is reacted with a silylating agent and base in a solvent that will allow the silylation to proceed. Preferred silylating agents include tert-butyldimethylsilyl chloride and tert-butyldimethylsilyl trifluoromethanesulphonate. The most preferred silylating agent is tert-butyldimethylsilyl trifluoromethanesulphonate. Preferred bases include triethylamine, trimethylamine, and 2,6-lutidine. More preferred bases include triethylamine and 2,6-lutidine. The most preferred base is 2,6-lutidine. Preferred solvents include halogenated hydrocarbon solvents with dichloromethane being the most preferred solvent. The reaction is allowed to proceed at a temperature of preferably -100°C to 100°C, more preferably -80°C to 80°C, and most preferably -70°C to 23°C.

The resulting silylated compound is isolated by methods known to those of ordinary skill in the art. Such methods include extraction, solvent evaporation, distillation, and crystallization. Preferably, the silyl ether is purified after isolation by distillation under vacuum.

The silylated compound is then reacted with the cuprate generated via Grignard formation of the appropriate alkenyl bromide as disclosed, for example, in the following references: H.O. House et. al., "The Chemistry of Carbanions: A Convenient Precursor for the Generation of Lithium Organocuprates", J. Org. Chem., Vol. 40, pp. 1460-69 (1975); and P. Knochel et. al., "Zinc and Copper Carbenoids as Efficient and Selective a'/d' Multicoupling Reagents", J. Amer. Chem. Soc., Vol. 111, p. 6474-76 (1989). Preferred alkenyl bromides include 4-bromo-1-butene, 4-bromo-1-butyne, 4-bromo-2-methyl-1-butene, and 4-bromo-2-ethyl-1-butene. The most preferred alkenyl bromide is 4-bromo-1-butene. Preferred solvents include ethereal solvents, of which diethyl ether and tetrahydrofuran are preferred. The most preferred solvent is tetrahydrofuran. The Grignard reagent is allowed to form at a temperature of 100°C to 23°C, more preferably

85°C to 30°C, and most preferably 75°C to 65°C. The reaction time is preferably 1 to 6 hours, more preferably 2 to 5 hours, and most preferably 3 to 4 hours.

Once the Grignard reagent is formed, the cuprate is generated from the alkenyl magnesium species. The temperature range for cuprate formation is -100°C and 0°C. The preferred temperature range is -80°C to -20°C, more preferably -75°C to -50°C. The preferred reaction time is 30 minutes to 6 hours, more preferably 45 minutes to 3 hours, and most preferably 1 to 1.5 hours.

The alkene thus formed is isolated by methods known to one of ordinary skill in the art. Such methods include, but are not limited to, extraction, solvent evaporation, distillation, and crystallization. Preferably, the alkene is purified by flash chromatography on silica gel (Merck, 230-400 mesh) using 10% EtOAc/hexanes as the eluent. The alkene is then reacted with a hydride reducing agent and a polar, protic solvent to give the C-9 alcohol. Preferred reducing agents include lithium aluminum hydride, sodium borohydride, and L-selectride. More preferred reducing agents include sodium borohydride, and L-selectride. The most preferred reducing agent is sodium borohydride. Preferred solvents include methanol, ethanol, and butanol. The most preferred solvent is methanol. The reduction is carried out at a temperature between -100°C and 23°C. The preferred temperature range is -60°C to 0°C. The most preferred temperature range is -45°C to -20°C.

The resulting alcohol is isolated by methods known to one of ordinary skill in the art. Such methods include, but are not limited to, extraction, solvent evaporation, distillation, and crystallization. Preferably, the alcohol is purified by flash chromatography on silica gel (Merck, 230-400 mesh) using 20% EtOAc/hexanes as the eluent.

The resultant alcohol can be protected as described previously herein. Preferred silylating agents in this case also include tert-butyldimethylsilyl chloride and tert-butyldimethylsilyl trifluoromethanesulfonate. The most preferred silylating agent is tert-butyldimethylsilyl trifluoromethanesulfonate. Preferred bases include triethylamine, trimethylamine, and 2,6-lutidine. More preferred bases include triethylamine and 2,6-lutidine. The most preferred base is 2,6-lutidine. Preferred solvents include halogenated

hydrocarbon solvents with dichloromethane being the most preferred solvent. The reaction is allowed to proceed at a temperature of preferably -100°C to 100°C, more preferably -80°C to 80°C, and most preferably -70°C to 23°C.

The resulting silylated compound is isolated by methods known to those of ordinary skill in the art. Such methods include, but are not limited to, extraction, solvent evaporation, distillation, and crystallization. Preferably, the silyl ether is purified after isolation by distillation under vacuum

The protected or alcohol is then treated with a form of osmium, and sodium periodate in a solvent where they are both soluble. Preferred forms of osmium include osmium tetraoxide and potassium osmate. Preferred solvent systems include 1:1 mixtures of acetic acid and water and 1:1:2 mixtures of water, acetic acid and THF. The result of this treatment is the aldehyde, **S4b**.

The compound **S4b** is isolated by methods known to one of ordinary skill in the art. Such methods include, but are not limited to, extraction, solvent evaporation, distillation, and crystallization. Preferably, **S4b** is purified by flash chromatography on silica gel (Merck, 230-400 mesh) using 20% EtOAc/hexanes as the eluent.

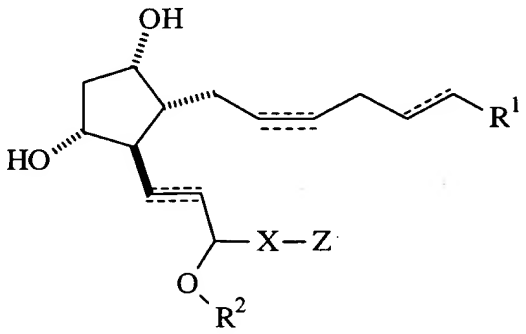
The key intermediate aldehyde depicted as **S4b** can be reacted with a variety unsaturated carbon nucleophiles to provide the C-9 and C-11-protected 13,14-dihydro-16-tetranor prostaglandin $F_{1\alpha}$ derivatives depicted as **S4c**.

With alkyne nucleophiles, the reaction is carried out preferably at -80°C to 0°C, more preferably -80°C to -20°C, and most preferably -80°C to -40°C. Preferred bases for the reaction include *n*-butyl lithium, *s*-butyl lithium, *t*-butyl lithium, and lithium diisopropyl amide ("LDA"). Preferred solvents for the reaction are ether solvents. Preferred solvents include diethyl ether, and tetrahydrofuran. The most preferred solvent is tetrahydrofuran. With heterocyclic nucleophiles, preferred solvents include ethereal solvents. More preferred ethereal solvents include diethyl ether, dibutyl ether and tetrahydrofuran. The most preferred ethereal solvent is tetrahydrofuran.

The resulting compounds depicted as **S4c** can then be deprotected using techniques known to one of ordinary skill in the art, and isolated yielding the 13,14-dihydro-15-substituted-15-pentanor prostaglandin $F_{1\alpha}$ derivatives depicted by **Formula VI**.

Compounds depicted by **Formula VII** can be made directly from the C-9 and C-11-protected 13,14-dihydro-16-tetranor prostaglandin $F_{1\alpha}$ derivatives depicted as **S4c** by methods known to one of ordinary skill in the art. For example, the condensation of methyl esters of **S4c** with amines or hydroxylamine provides compounds depicted by **Formula VII**. These compounds are isolated by methods known to one of ordinary skill in the art. Such methods include extraction, solvent evaporation, distillation, and crystallization.

Examples of PGF's having the structure:



which are suitable for component A) include: cloprostenol (estrumate), fluprostenol (equimate), tiaprost, alfaprostol, delprostenate, froxiprost, 9-alpha, 11-alpha, 15-alpha-trihydroxy-16-(3-chlorophenoxy)-omega-tetranor-prosta-4-cis-13-trans-dienoic acid, latanoprost and their analogs; and 13,14-dihydro-16-((3-trifluoromethyl)phenoxy)-16-tetranor prostaglandin $F_{1\alpha}$, 17-((3-trifluoromethyl)phenyl)-17-trinor-prostaglandin $F_{2\alpha}$ and its analogs, 13,14-dihydro-18-thienyl-18-dinor prostaglandin $F_{1\alpha}$ and their analogs. Additional PGF's are also disclosed in CRC Handbook of Eicosanoids: Prostaglandins and Related Lipids, Volume I, Chemical and Biochemical Aspects, Part B. Ed. by Anthony L. Willis, CRC Press, Boca Raton, Table Four, pp. 80-97 (1987) and references therein.

Preferred PGF's of the present invention are further selective for the FP receptor over an excitatory prostaglandin receptor in a ratio of 1:10, preferably from 1:20, more preferably from 1:50.

Compositions of the Invention

This invention further relates to a composition for treating hair loss. "Treating hair loss" means arresting hair loss, reversing hair loss, or both, and promoting hair growth. The composition comprises component A) the PGF described above and component B) a

carrier. The composition may further comprise component C) one or more optional activity enhancers.

The composition can be a pharmaceutical or cosmetic composition, administered for treatment or prophylaxis of hair loss. Standard pharmaceutical formulation techniques are used, such as those disclosed in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa (1990).

The composition further comprises component B) a carrier. "Carrier" means one or more compatible substances that are suitable for administration to a mammal. Carrier includes solid or liquid diluents, hydrotopes, surface-active agents, and encapsulating substances. "Compatible" means that the components of the composition are capable of being commingled with the PGF's, and with each other, in a manner such that there is no interaction which would substantially reduce the efficacy of the composition under ordinary use situations. Carriers must be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the mammal being treated. The carrier can be inert, or it can possess pharmaceutical benefits, cosmetic benefits, or both.

The choice of carrier for component B) depends on the route by which A) the PGF will be administered and the form of the composition. The composition may be in a variety of forms, suitable, for example, for systemic administration (e.g., oral, rectal, nasal, sublingual, buccal, or parenteral) or topical administration (e.g., local application on the skin, ocular, liposome delivery systems, or iontophoresis). Topical administration directly to the locus of desired hair growth is preferred.

Carriers for systemic administration typically comprise one or more ingredients selected from the group consisting of a) diluents, b) lubricants, c) binders, d) disintegrants, e) colorants, f) flavors, g) sweeteners, h) antioxidants, j) preservatives, k) glidants, m) solvents, n) suspending agents, o) surfactants, combinations thereof, and others.

Ingredient a) is a diluent. Suitable diluents include sugars such as glucose, lactose, dextrose, and sucrose; polyols such as propylene glycol; calcium carbonate; sodium carbonate; glycerin; mannitol; sorbitol; and maltodextrin.

Ingredient b) is a lubricant. Suitable lubricants are exemplified by solid lubricants including silica, talc, stearic acid and its magnesium salts and calcium salts, calcium sulfate; and liquid lubricants such as polyethylene glycol and vegetable oils such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma.

Ingredient c) is a binder. Suitable binders include polyvinylpyrrolidone; magnesium aluminum silicate; starches such as corn starch and potato starch; gelatin; tragacanth; and cellulose and its derivatives, such as sodium carboxymethylcellulose, ethylcellulose, methylcellulose, microcrystalline cellulose, and hydroxypropylmethylcellulose; carbomer; providone; acacia; guar gum; and xanthan gum.

Ingredient d) is a disintegrant. Suitable disintegrants include agar, alginic acid and the sodium salt thereof, effervescent mixtures, croscarmelose, crospovidone, sodium carboxymethyl starch, sodium starch glycolate, clays, and ion exchange resins.

Ingredient e) is a colorant such as an FD&C dye.

Ingredient f) is a flavor such as menthol, peppermint, and fruit flavors.

Ingredient g) is a sweetener such as saccharin and aspartame.

Ingredient h) is an antioxidant such as butylated hydroxyanisole, butylated hydroxytoluene, and vitamin E.

Ingredient j) is a preservative such as phenol, alkyl esters of parahydroxybenzoic acid, benzoic acid and the salts thereof, boric acid and the salts thereof, sorbic acid and the salts thereof, chorbutanol, benzyl alcohol, thimerosal, phenylmercuric acetate and nitrate, nitromersol, benzalkonium chloride, cetylpyridinium chloride, methyl paraben, and propyl paraben. Particularly preferred are the salts of benzoic acid, cetylpyridinium chloride, methyl paraben and propyl paraben, and sodium benzoate.

Ingredient k) is a glidant such as silicon dioxide.

Ingredient m) is a solvent, such as water, isotonic saline, ethyl oleate, alcohols such as ethanol, glycerin, glycols (e.g., polypropylene glycol and polyethylene glycol), and buffer solutions (e.g., phosphate, potassium acetate, boric carbonic, phosphoric, succinic, malic, tartaric, citric, acetic, benzoic, lactic, glyceric, gluconic, glutaric and glutamic).

Ingredient n) is a suspending agent. Suitable suspending agents include AVICEL® RC-591 from FMC Corporation of Philadelphia, Pennsylvania and sodium alginate.

Ingredient o) is a surfactant such as lecithin, polysorbate 80, sodium lauryl sulfate, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene monoalkyl ethers, sucrose monoesters, lanolin esters, and lanolin ethers. Suitable surfactants are known in the art and commercially available, e.g., the TWEENS® from Atlas Powder Company of Wilmington, Delaware.

Compositions for parenteral administration typically comprise A) 0.1 to 10% of a PGF and B) 90 to 99.9% of a carrier comprising a) a diluent, and m) a solvent. Preferably, component a) is propylene glycol and m) is ethanol or ethyl oleate.

Compositions for oral administration can have various dosage forms. For example, solid forms include tablets, capsules, granules, and bulk powders. These oral dosage forms comprise a safe and effective amount, usually at least 5%, and preferably from 25% to 50%, of A) the PGF. The oral dosage compositions further comprise B) 50 to 95% of a carrier, preferably 50 to 75%.

Tablets can be compressed, tablet triturates, enteric-coated, sugar-coated, film-coated, or multiple-compressed. Tablets typically comprise A) the PGF, and B) a carrier comprising ingredients selected from the group consisting of a) diluents, b) lubricants, c) binders, d) disintegrants, e) colorants, f) flavors, g) sweeteners, k) glidants, and combinations thereof. Preferred diluents include calcium carbonate, sodium carbonate, mannitol, lactose and cellulose. Preferred binders include starch, gelatin, and sucrose. Preferred disintegrants include alginic acid, and croscarmelose. Preferred lubricants include magnesium stearate, stearic acid, and talc. Preferred colorants are the FD&C dyes, which can be added for appearance. Chewable tablets preferably contain g) sweeteners such as aspartame and saccharin, or f) flavors such as menthol, peppermint, and fruit flavors.

Capsules (including time release and sustained release formulations) typically comprise A) the PGF, and B) a carrier comprising one or more a) diluents disclosed above in a capsule comprising gelatin. Granules typically comprise A) the PGF, and preferably further comprise k) glidants such as silicon dioxide to improve flow characteristics.

The selection of ingredients in the carrier for oral compositions depends on secondary considerations like taste, cost, and shelf stability, which are not critical for the purposes of this invention. One skilled in the art can optimize appropriate ingredients without undue experimentation.

The solid compositions may also be coated by conventional methods, typically with pH or time-dependent coatings, such that A) the PGF is released in the gastrointestinal tract at various times to extend the desired action. The coatings typically comprise one or more components selected from the group consisting of cellulose acetate phthalate, polyvinylacetate phthalate, hydroxypropyl methyl cellulose phthalate, ethyl cellulose, acrylic resins such as EUDRAGIT® coatings (available from Rohm & Haas G.M.B.H. of Darmstadt, Germany), waxes, shellac, polyvinylpyrrolidone, and other commercially available film-coating preparations such as Dri-Klear, manufactured by Crompton & Knowles Corp., Mahwah, NJ or OPADRY® manufactured by Colorcon, Inc., of West Point, Pennsylvania.

Compositions for oral administration can also have liquid forms. For example, suitable liquid forms include aqueous solutions, emulsions, suspensions, solutions reconstituted from non-effervescent granules, suspensions reconstituted from non-effervescent granules, effervescent preparations reconstituted from effervescent granules, elixirs, tinctures, syrups, and the like. Liquid orally administered compositions typically comprise A) the PGF and B) a carrier comprising ingredients selected from the group consisting of a) diluents, e) colorants, and f) flavors, g) sweeteners, j) preservatives, m) solvents, n) suspending agents, and o) surfactants. *Peroral* liquid compositions preferably comprise one or more ingredients selected from the group consisting of e) colorants, f) flavors, and g) sweeteners.

Other compositions useful for attaining systemic delivery of the subject compounds include sublingual, buccal and nasal dosage forms. Such compositions typically comprise one or more of soluble filler substances such as a) diluents including sucrose, sorbitol and mannitol; and c) binders such as acacia, microcrystalline cellulose, carboxymethylcellulose, and hydroxypropylmethylcellulose. Such compositions may

further comprise b) lubricants, e) colorants, f) flavors, g) sweeteners, h) antioxidants, and k) glidants.

The compositions may further comprise component C) an optional activity enhancer. Component C) is preferably selected from the group consisting of i) hair growth stimulants (other than the PGF) and ii) penetration enhancers.

Component i) is an optional hair growth stimulant. Component i) is exemplified by vasodilators, antiandrogens, cyclosporins, cyclosporin analogs, antimicrobials, anti-inflammatories, thyroid hormones, thyroid hormone derivatives, and thyroid hormone analogs, non-selective prostaglandin agonists or antagonists, retinoids, triterpenes, combinations thereof, and others. "Non-selective prostaglandin" agonists and antagonists differ from component A) in that they do not selectively activate the FP receptor, and they may activate other receptors.

Vasodilators such as potassium channel agonists including minoxidil and minoxidil derivatives such as aminexil and those described in U.S. Patent Numbers 3,382,247, 5,756,092, 5,772,990, 5,760,043, 5,466,694, 5,438,058, 4,973,474, and cromakalin and diazoxide can be used as optional hair growth stimulants in the composition.

Examples of suitable antiandrogens include 5-α-reductase inhibitors such as finasteride and those described in U.S. Patent Number 5,516,779, and in Nane et al., Cancer Research 58, "Effects of Some Novel Inhibitors of C17,20-Lyase and 5α-Reductase *in vitro* and *in vivo* and Their Potential Role in the Treatment of Prostate Cancer," as well as cyproterone acetate, azelaic acid and its derivatives and those compounds described in U.S. Patent Number 5,480,913, flutamide, and those compounds described in U.S. Patent Numbers 5,411,981, 5,565,467, and 4,910,226.

Antimicrobials include selenium sulfide, ketoconazole, triclocarbon, triclosan, zinc pyrithione, itraconazole, asiatic acid, hinokitiol, mipirocin and those described in EPA 0,680,745, clinacycin hydrochloride, benzoyl peroxide, benzyl peroxide and minocyclin.

Examples of suitable anti-inflammatories include glucocorticoids such as hydrocortisone, mometasone furoate and prednisolone, nonsteroidal anti-inflammatories

including cyclooxygenase or lipoxygenase inhibitors such as those described in U.S. Patent Number 5,756,092, and benzydamine, salicylic acid, and those compounds described in EPA 0,770,399, published May 2, 1997, WO 94/06434, published March 31, 1994, and FR 2,268,523, published November 21, 1975.

3,5,3'-Triiodothyronine is an example of a suitable thyroid hormone.

Examples of suitable non-selective prostaglandins agonists and antagonists include compounds such as those described in WO 98/33497, Johnstone, published August 6, 1998, WO 95/11003, Stjernschantz, published April 27, 1995, JP 97-100091, Ueno and JP 96-134242, Nakamura.

Suitable retinoids include isotretinoin, acitretin, and tazarotene.

Other optional hair growth stimulants for component i) include benzalkonium chloride, benzethonium chloride, phenol, estradiol, chlorpheniramine maleate, chlorophyllin derivatives, cholesterol, salicylic acid, cysteine, methionine, red pepper tincture, benzyl nicotinate, D,L - menthol, peppermint oil, calcium pantothenate, panthenol, castor oil, prednisolone, resorcinol, chemical activators of protein kinase C, glycosaminoglycan chain cellular uptake inhibitors, inhibitors of glycosidase activity, glycosaminoglycanase inhibitors, esters of pyroglutamic acid, hexosaccharic acids or acylated hexosaccharic acids, aryl-substituted ethylenes, N-acylated amino acids, flavinoids, ascomycin derivatives and analogs, histamine antagonists such as diphenhydramine hydrochloride, triterpenes such as oleanolic acid and ursolic acid and those described in U.S. Patent Numbers 5,529,769, 5,468,888, 5,631,282, and 5,679,705, JP 10017431, WO 95/35103, JP 09067253, WO 92/09262, JP 62093215, and JP 08193094; saponins such as those described in EP 0,558,509 to Bonte et al., published September 8, 1993 and WO 97/01346 to Bonte et al, published January 16, 1997, proteoglycanase or glycosaminoglycanase inhibitors such as those described in U.S. Patent Numbers 5,015,470, 5,300,284, and 5,185,325, estrogen agonists and antagonists, pseudoterins, cytokine and growth factor promoters, analogs or inhibitors such as interleukin1 inhibitors, interleukin-6 inhibitors, interleukin-10 promoters, and tumor necrosis factor inhibitors, vitamins such as vitamin D analogs and parathyroid hormone antagonists, Vitamin B12 analogs and panthenol, interferon agonists and antagonists,

hydroxyacids such as those described in U.S. Patent Number 5,550,158, benzophenones, and hydantoin anticonvulsants such as phenytoin, and combinations thereof.

Other additional hair growth stimulants are described in JP 09-157,139 to Tsuji et al., published June 17, 1997; EP 0277455 A1 to Mirabeau, published August 10, 1988; WO 97/05887 to Cabo Soler et al., published February 20, 1997; WO 92/16186 to Bonte et al., published March 13, 1992; JP 62-93215 to Okazaki et al., published April 28, 1987; U.S. Patent 4,987,150 to Kurono et al., issued January 22, 1991; JP 290811 to Ohba et al., published October 15, 1992; JP 05-286,835 to Tanaka et al., published November 2, 1993, FR 2,723,313 to Greff, published August 2, 1994, U. S. Patent Number 5,015,470 to Gibson, issued May 14, 1991, U.S. Patent Number 5,559,092, issued September 24, 1996, U.S. Patent Number 5,536,751, issued July 16, 1996, U.S. Patent Number 5,714,515, issued February 3, 1998, EPA 0,319,991, published June 14, 1989, EPA 0,357,630, published October 6, 1988, EPA 0,573,253, published December 8, 1993, JP 61-260010, published November 18, 1986, U.S. Patent Number 5,772,990, issued June 30, 1998, U.S. Patent Number 5,053, 410, issued October 1, 1991, and U.S. Patent Number 4,761,401, issued August 2, 1988.

The most preferred activity enhancers are minoxidil and finasteride, most preferably minoxidil.

Component ii) is a penetration enhancer that can be added to all of the compositions for systemic administration. The amount of component ii), when present in the composition, is typically 1 to 5 %. Examples of penetration enhancers include 2-methyl propan-2-ol, propan-2-ol, ethyl-2-hydroxypropanoate, hexan-2,5-diol, polyoxyethylene(2) ethyl ether, di(2-hydroxypropyl) ether, pentan-2,4-diol, acetone, polyoxyethylene(2) methyl ether, 2-hydroxypropionic acid, 2-hydroxyoctanoic acid, propan-1-ol, 1,4-dioxane, tetrahydrofuran, butan-1,4-diol, propylene glycol dipelargonate, polyoxypropylene 15 stearyl ether, octyl alcohol, polyoxyethylene ester of oleyl alcohol, oleyl alcohol, lauryl alcohol, dioctyl adipate, dicapryl adipate, di-isopropyl adipate, di-isopropyl sebacate, dibutyl sebacate, diethyl sebacate, dimethyl sebacate, dioctyl sebacate, dibutyl suberate, dioctyl azelate, dibenzyl sebacate, dibutyl phthalate, dibutyl azelate, ethyl myristate, dimethyl azelate, butyl myristate, dibutyl succinate, didecyl phthalate,

decyl oleate, ethyl caproate, ethyl salicylate, isopropyl palmitate, ethyl laurate, 2-ethyl-hexyl pelargonate, isopropyl isostearate, butyl laurate, benzyl benzoate, butyl benzoate, hexyl laurate, ethyl caprate, ethyl caprylate, butyl stearate, benzyl salicylate, 2-hydroxypropanoic acid, 2-hydroxyoctanoic acid, dimethyl sulphoxide, N,N-dimethyl acetamide, N,N-dimethyl formamide, 2-pyrrolidone, 1-methyl-2-pyrrolidone, 5-methyl-2-pyrrolidone, 1,5-dimethyl-2-pyrrolidone, 1-ethyl-2-pyrrolidone, phosphine oxides, sugar esters, tetrahydrofurfural alcohol, urea, diethyl-m-toluamide, 1-dodecylazacycloheptan-2-one, omega three fatty acids and fish oils, and combinations thereof.

In a preferred embodiment of the invention, the PGF's are topically administered. Topical compositions that can be applied locally to the skin may be in any form including solutions, oils, creams, ointments, gels, lotions, shampoos, leave-on and rinse-out hair conditioners, milks, cleansers, moisturizers, sprays, skin patches, and the like. Topical compositions comprise: component A) the PGF described above and component B) a carrier. The carrier of the topical composition preferably aids penetration of the PGF's into the skin to reach the environment of the hair follicle. Topical compositions preferably further comprise C) one or more of the optional activity enhancers described above.

The exact amounts of each component in the topical composition depend on various factors. The amount of component A) depends on the IC_{50} of the PGF selected. "IC_{50}" means inhibitory concentration 50th percentile. The amount of component A) added to the topical composition is:

$$IC_{50} \times 10^{-2} \geq \%\text{ of component A)} \geq IC_{50} \times 10^{-3},$$

where IC_{50} is expressed in nanomolar units. For example, if the IC_{50} of the PGF is 1 nM, the amount of component A) will be 0.001 to 0.01%. If the IC_{50} of the PGF is 10 nM, the amount of component A) will be 0.01 to 0.1%. If the IC_{50} of the PGF is 100 nM, the amount of component A) will be 0.1 to 1.0%. If the IC_{50} of the PGF is 1000 nM, the amount of component A) will be 1.0 to 10%, preferably 1.0 to 5%. If the amount of component A) is outside the ranges specified above (i.e., either higher or lower), efficacy of the treatment may be reduced. IC_{50} can be calculated according to the method in Reference Example 1, below. One skilled in the art can calculate IC_{50} without undue experimentation.

The topical composition preferably further comprises 1 to 20% component C), and a sufficient amount of component B) such that the amounts of components A), B), and C), combined equal 100%. The amount of B) the carrier employed in conjunction with the PGF is sufficient to provide a practical quantity of composition for administration per unit dose of the compound. Techniques and compositions for making dosage forms useful in the methods of this invention are described in the following references: Modern Pharmaceutics, Chapters 9 and 10, Banker & Rhodes, eds. (1979); Lieberman et al., Pharmaceutical Dosage Forms: Tablets (1981); and Ansel, Introduction to Pharmaceutical Dosage Forms, 2nd Ed., (1976).

Component B) the carrier may comprise a single ingredient or a combination of two or more ingredients. In the topical compositions, component B) is a topical carrier. Preferred topical carriers comprise one or more ingredients selected from the group consisting of water, alcohols, aloe vera gel, allantoin, glycerin, vitamin A and E oils, mineral oil, propylene glycol, polypropylene glycol-2 myristyl propionate, dimethyl isosorbide, combinations thereof, and the like. More preferred carriers include propylene glycol, dimethyl isosorbide, and water.

The topical carrier may comprise one or more ingredients selected from the group consisting of q) emollients, r) propellants, s) solvents, t) humectants, u) thickeners, v) powders, and w) fragrances in addition to, or instead of, the preferred topical carrier ingredients listed above. One skilled in the art would be able to optimize carrier ingredients for the topical compositions without undue experimentation.

Ingredient q) is an emollient. The amount of ingredient q) in the topical composition is typically 5 to 95%. Suitable emollients include stearyl alcohol, glyceryl monoricinoleate, glyceryl monostearate, propane-1,2-diol, butane-1,3-diol, mink oil, cetyl alcohol, isopropyl isostearate, stearic acid, isobutyl palmitate, isocetyl stearate, oleyl alcohol, isopropyl laurate, hexyl laurate, decyl oleate, octadecan-2-ol, isocetyl alcohol, cetyl palmitate, di-n-butyl sebacate, isopropyl myristate, isopropyl palmitate, isopropyl stearate, butyl stearate, polyethylene glycol, triethylene glycol, lanolin, sesame oil, coconut oil, arachis oil, castor oil, acetylated lanolin alcohols, petrolatum, mineral oil, butyl myristate, isostearic acid, palmitic acid, isopropyl linoleate, lauryl lactate, myristyl

lactate, decyl oleate, myristyl myristate, polydimethylsiloxane, and combinations thereof. Preferred emollients include stearyl alcohol and polydimethylsiloxane.

Ingredient r) is a propellant. The amount of ingredient r) in the topical composition is typically 5 to 95%. Suitable propellants include propane, butane, isobutane, dimethyl ether, carbon dioxide, nitrous oxide, and combinations thereof.

Ingredient s) is a solvent. The amount of ingredient s) in the topical composition is typically 5 to 95 %. Suitable solvents include water, ethyl alcohol, methylene chloride, isopropanol, castor oil, ethylene glycol monoethyl ether, diethylene glycol monobutyl ether, diethylene glycol monoethyl ether, dimethylsulfoxide, dimethyl formamide, tetrahydrofuran, and combinations thereof. Preferred solvents include ethyl alcohol.

Ingredient t) is a humectant. The amount of ingredient t) in the topical composition is typically 5 to 95 %. Suitable humectants include glycerin, sorbitol, sodium 2-pyrrolidone-5-carboxylate, soluble collagen, dibutyl phthalate, gelatin, and combinations thereof. Preferred humectants include glycerin.

Ingredient u) is a thickener. The amount of ingredient u) in the topical composition is typically 0 to 95%.

Ingredient v) is a powder. The amount of ingredient v) in the topical composition is typically 0 to 95 %. Suitable powders include chalk, talc, fullers earth, kaolin, starch, gums, colloidal silicon dioxide, sodium polyacrylate, tetra alkyl ammonium smectites, trialkyl aryl ammonium smectites, chemically modified magnesium aluminum silicate, organically modified montmorillonite clay, hydrated aluminum silicate, fumed silica, carboxyvinyl polymer, sodium carboxymethyl cellulose, ethylene glycol monostearate, and combinations thereof.

Ingredient w) is a fragrance. The amount of ingredient w) in the topical composition is typically 0.001 to 0.5%, preferably 0.001 to 0.1%.

Component C) the optional activity enhancer is as described above. Any of the i) hair growth stimulants and ii) penetration enhancers may be added to the topical compositions. Preferably, the topical composition comprises 0.01 to 15% of component i) the optional hair growth stimulant. More preferably, the composition comprises 0.1 to

10%, and most preferably 0.5 to 5% of component i). Preferably, the topical composition comprises 1 to 5% of component ii).

In an alternative embodiment of the invention, topical pharmaceutical compositions for ocular administration are prepared by conventional methods. Topical pharmaceutical compositions for ocular administration typically comprise A) a PGF, B) a carrier, such as purified water, and one or more ingredients selected from the group consisting of y) sugars such as dextrans, particularly dextran 70, z) cellulose or a derivative thereof, aa) a salt, bb) disodium EDTA (Edetate disodium), and cc) a pH adjusting additive.

Examples of z) cellulose derivatives suitable for use in the topical pharmaceutical composition for ocular administration include sodium carboxymethyl cellulose, ethyl cellulose, methyl cellulose, and hydroxypropylmethylcellulose. Hydroxypropylmethylcellulose is preferred.

Examples of aa) salts suitable for use in the for use in the topical pharmaceutical composition for ocular administration include sodium chloride, potassium chloride, and combinations thereof.

Examples of cc) pH adjusting additives include HCl or NaOH in amounts sufficient to adjust the pH of the topical pharmaceutical composition for ocular administration to 7.2-7.5.

This invention further relates to a method for darkening hair, thickening hair, and reversing hair graying. The method comprises applying the topical composition for treating hair loss to hair, to skin in the locus of hair, or both. For example, the topical composition may be applied to hair growing on the scalp or eyelashes. The topical composition can be, for example, a cosmetic composition prepared as described above. An example of a composition that may be applied to eyelashes is a mascara. The PGF may be added to mascara compositions known in the art, such as the mascara described in U.S. Patent No. 5,874,072, which is hereby incorporated by reference. The mascara further comprises dd) a water-insoluble material, ee) a water-soluble, film-forming polymer, ff) a wax, o) a surfactant, gg) a pigment, and s) a solvent.

Ingredient dd) is a water-insoluble material selected from the group consisting of acrylate copolymers; styrene/acrylate/methacrylate copolymers; acrylic latex; styrene/acrylic ester copolymer latex; polyvinylacetate latex; vinyl acetate/ethylene copolymer latex; styrene/butadiene copolymer latex; polyurethane latex; butadiene/acrylonitrile copolymer latex; styrene/acrylate/acrylonitrile copolymer latex; and mixtures thereof, wherein the acrylate copolymers, and the styrene/acrylate/methacrylate copolymers additionally comprise ammonia, propylene glycol, a preservative and a surfactant.

Ingredient ee) is a water-soluble, film-forming polymer. Ingredient ee) is selected from the group consisting of vinyl alcohol/poly(alkyleneoxy)acrylate, vinyl alcohol/vinyl acetate/poly-(alkyleneoxy)acrylate, polyethylene oxide, polypropylene oxide, acrylates/octyl-acrylamide copolymers and mixtures thereof.

Ingredient ff) is a wax. "Wax" means a lower-melting organic mixture or compound of high molecular weight, solid at room temperature and generally similar in composition to fats and oils except that they contain no glycerides. Some are hydrocarbons, others are esters of fatty acids and alcohols. Waxes useful in this invention are selected from the group consisting of animal waxes, vegetable waxes, mineral waxes, various fractions of natural waxes, synthetic waxes, petroleum waxes, ethylenic polymers, hydrocarbon types such as Fischer-Tropsch waxes, silicone waxes, and mixtures thereof wherein the waxes have a melting point between 55 and 100°C.

Ingredient o) is surfactant, as described above. Ingredient o) in the mascara is preferably a surfactant having an HLB from 3 to 15. Suitable surfactants include those disclosed in the C.T.F.A. Cosmetic Ingredient Handbook, pp. 587-592 (1992); Remington's Pharmaceutical Sciences, 15th ed., pp. 335-337 (1975); and McCutcheon's Volume 1, Emulsifiers & Detergents, North American Edition, pp. 236-239 (1994).

Ingredient gg) is a pigment. Suitable pigments include inorganic pigments, organic lake pigments, pearlescent pigments, and mixtures thereof. Inorganic pigments useful in this invention include those selected from the group consisting of rutile or anatase titanium dioxide, coded in the Color Index under the reference CI 77,891; black, yellow, red and brown iron oxides, coded under references CI 77,499, 77,492 and, 77,491;

manganese violet (CI 77,742); ultramarine blue (CI 77,007); chromium oxide (CI 77,288); chromium hydrate (CI 77,289); and ferric blue (CI 77,510); and mixtures thereof.

The organic pigments and lakes useful in this invention include those selected from the group consisting of D&C Red No. 19 (CI 45,170), D&C Red No. 9 (CI 15,585), D&C Red No. 21 (CI 45,380), D&C Orange No. 4 (CI 15,510), D&C Orange No. 5 (CI 45,370), D&C Red No. 27 (CI 45,410), D&C Red No. 13 (CI 15,630), D&C Red No. 7 (CI 15,850), D&C Red No. 6 (CI 15,850), D&C Yellow No. 5 (CI 19,140), D&C Red No. 36 (CI 12,085), D&C Orange No. 10 (CI 45,425), D&C Yellow No. 6 (CI 15,985), D&C Red No. 30 (CI 73,360), D&C Red No. 3 (CI 45,430), and the dye or lakes based on Cochineal Carmine (CI 75,570), and mixtures thereof.

The pearlescent pigments useful in this invention include those selected from the group consisting of the white pearlescent pigments such as mica coated with titanium oxide, bismuth oxychloride, colored pearlescent pigments such as titanium mica with iron oxides, titanium mica with ferric blue, chromium oxide and the like, titanium mica with an organic pigment of the above-mentioned type as well as those based on bismuth oxychloride and mixtures thereof.

Ingredient s) is a solvent described above, preferably water.

The amount of A) the PGF added to the mascara is as described above for topical compositions.

The PGF's may also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines. A preferred formulation for topical delivery of the present compounds uses liposomes as described in Dowton et al., "Influence of Liposomal Composition on Topical Delivery of Encapsulated Cyclosporin A: I. An *in vitro* Study Using Hairless Mouse Skin", S.T.P. Pharma Sciences, Vol. 3, pp. 404 - 407 (1993); Wallach and Philippot, "New Type of Lipid Vesicle: Novasome®", Liposome Technology, Vol. 1, pp. 141 - 156 (1993); Wallach, U.S. Patent No. 4,911,928, assigned to Micro-Pak, Inc., issued March 27, 1990; and Weiner et al., U.S. Patent No. 5,834,014, assigned to The University of Michigan and Micro-Pak, Inc., issued November 10, 1998

(with respect to Weiner et al., with a compound as described herein administered in lieu of, or in addition to, minoxidil).

The PGF's may also be administered by iontophoresis. See, e.g., Internet site www.unipr.it/arpa/dipfarm/erasmus/erasm14.html; Banga et al., "Hydrogel-based Iontotherapeutic Delivery Devices for Transdermal Delivery of Peptide/Protein Drugs", Pharm. Res., Vol. 10 (5), pp. 697-702 (1993); Ferry, "Theoretical Model of Iontophoresis Utilized in Transdermal Drug Delivery", Pharmaceutical Acta Helvetiae, Vol 70, pp. 279-287 (1995); Gangarosa et al., "Modern Iontophoresis for Local Drug Delivery", Int. J. Pharm., Vol. 123, pp. 159-171 (1995); Green et al., "Iontophoretic Delivery of a Series of Tripeptides Across the Skin *in vitro*", Pharm. Res., Vol 8, pp. 1121-1127 (1991); Jadoul et al., "Quantification and Localization of Fentanyl and TRH Delivered by Iontophoresis in the Skin", Int. J. Pharm., Vol. 120, pp. 221-8 (1995); O'Brien et al., "An Updated Review of its Antiviral Activity, Pharmacokinetic Properties and Therapeutic Efficacy", Drugs, Vol. 37, pp. 233-309 (1989); Parry et al., "Acyclovir Biovailability in Human Skin", J. Invest. Dermatol., Vol. 98 (6), pp. 856-63 (1992); Santi et al., "Drug Reservoir Composition and Transport of Salmon Calcitonin in Transdermal Iontophoresis", Pharm. Res., Vol 14 (1), pp. 63-66 (1997); Santi et al., "Reverse Iontophoresis - Parameters Determining Electroosmotic Flow: I. pH and Ionic Strength", J. Control. Release, Vol. 38, pp. 159-165 (1996); Santi et al., "Reverse Iontophoresis - Parameters Determining Electroosmotic Flow: II. Electrode Chamber Formulation", J. Control. Release, Vol. 42, pp. 29-36 (1996); Rao et al., "Reverse Iontophoresis: Noninvasive Glucose Monitoring *in vivo* in Humans", Pharm. Res., Vol. 12 (12), pp. 1869-1873 (1995); Thysman et al., "Human Calcitonin Delivery in Rats by Iontophoresis", J. Pharm. Pharmacol., Vol. 46, pp. 725-730 (1994); and Volpato et al., "Iontophoresis Enhances the Transport of Acyclovir through Nude Mouse Skin by Electrorepulsion and Electroosmosis", Pharm. Res., Vol. 12 (11), pp. 1623-1627 (1995).

The PGF's may be included in kits comprising a PGF, a systemic or topical composition described above, or both; and information, instructions, or both that use of the kit will provide treatment for hair loss in mammals (particularly humans). The information and instructions may be in the form of words, pictures, or both, and the like.

In addition or in the alternative, the kit may comprise a PGF, a composition, or both; and information, instructions, or both, regarding methods of application of the PGF or composition, preferably with the benefit of treating hair loss in mammals.

Methods of the Invention

This invention further relates to a method for treating hair loss in mammals. The method comprises administering to a mammal (preferably a human) suffering from hair loss, a PGF described above. For example, a mammal diagnosed with alopecia including male pattern baldness and female pattern baldness can be treated by the methods of this invention. Preferably, a systemic or topical composition comprising A) the PGF and B) a carrier is administered to the mammal. More preferably, the composition is a topical composition comprising A) the PGF, B) the carrier, and C) an optional activity enhancer.

The dosage of the PGF administered depends on the method of administration. For systemic administration, (e.g., oral, rectal, nasal, sublingual, buccal, or parenteral), typically, 0.5 mg to 300 mg, preferably 0.5 mg to 100 mg, more preferably 0.1 mg to 10 mg, of a PGF described above is administered per day. These dosage ranges are merely exemplary, and daily administration can be adjusted depending on various factors. The specific dosage of the PGF to be administered, as well as the duration of treatment, and whether the treatment is topical or systemic are interdependent. The dosage and treatment regimen will also depend upon such factors as the specific PGF used, the treatment indication, the efficacy of the compound, the personal attributes of the subject (such as, for example, weight, age, sex, and medical condition of the subject), compliance with the treatment regimen, and the presence and severity of any side effects of the treatment.

For topical administration (e.g., local application on the skin, ocular, liposome delivery systems, or iontophoresis), the topical composition is typically administered once per day. The topical compositions are administered daily for a relatively short amount of time (i.e., on the order of weeks). Generally, 6 to 12 weeks is sufficient. The topical compositions are preferably leave-on compositions. In general, the topical composition should not be removed for at least several hours after administration.

In addition to the benefits in treating hair loss, the inventors have surprisingly found that the PGF's in the compositions and methods of this invention also darken and thicken hair and may reverse hair graying. This invention further relates to a method for darkening and thickening hair. The method comprises applying the topical composition for treating hair loss to growing hair and skin in the locus of the growing hair. In a preferred embodiment of the invention, the topical composition, such as the mascara composition described above, is applied to eyelashes.

EXAMPLES

These examples are intended to illustrate the invention to those skilled in the art and should not be interpreted as limiting the scope of the invention set forth in the claims.

Reference Example 1 - Radioligand Binding Assay

IC_{50} of a PGF can be determined relative to $PGF_{2\alpha}$ using the Radioligand Binding Assay. As a control, the IC_{50} for $PGF_{2\alpha}$ itself should be no lower than 1.0 nM and no higher than 5.0 nM.

In this assay, COS-7 cells are transiently transfected with the hFP recombinant plasmid using LipofectAMINE Reagent. Forty-eight hours later, the tranfected cells are washed with Hank's Balanced Salt Solution (HBSS, without $CaCl_2$, $MgCl_2$, $MgSO_4$, or phenol red). The cells are detached with versene, and HBSS is added. The mixture is centrifuged at 200*g* for 10 minutes, at 4°C to pellet the cells. The pellet is resuspended in Phosphate-Buffered Saline-EDTA buffer (PBS; 1 mM EDTA; pH 7.4; 4°C). The cells are disrupted by nitrogen cavitation (Parr model 4639), at 800 psi, for 15 minutes at 4°C. The mixture is centrifuged at 1000*g* for 10 minutes at 4°C. The supernatant is centrifuged at 100,000*g* for 60 minutes at 4°C. The pellet is resuspended to 1 mg protein/mL TME buffer (50 mM Tris; 10 mM MgCl2; 1 mM EDTA; pH 6.0; 4°C) based on protein levels measured using the Pierce BCA Protein Assay kit. The homogenate is mixed for 10 seconds using a Kinematica POLYTRON ® (available from KINEMATICA AG,

Luzernerstrasse147A CH-6014 Littau, Switzerland). The membrane preparations are then stored at -80°C, until thawed for assay use.

The receptor competition binding assays are developed in a 96 well format. Each well contains 100 g of hFP membrane, 5 nM (3 H) PGF2, and the various competing compounds in a total volume of 200 L. The plates are incubated at 23°C for 1 hour. The incubation is terminated by rapid filtration using the Packard Filtermate 196 harvester through Packard UNIFILTER® GF/B filters (available from Packard Instrument Co., Inc. of Downers Grove Illinois) pre-wetted with TME buffer. The filter is washed four times with TME buffer. Packard Microscint 20, a high efficiency liquid scintillation cocktail, is added to the filter plate wells and the plates remain at room temperature for three hours prior to counting. The plates are read on a Packard TOPCOUNT® Microplate Scintillation Counter (also available from Packard Instrument Co., Inc.)

Reference Example 2 - Telogen Conversion Assay

PGF's are tested for their potential to grow hair using the Telogen Conversion Assay. The Telogen Conversion Assay measures the potential of a PGF to convert mice in the resting stage of the hair growth cycle ("telogen"), to the growth stage of the hair growth cycle ("anagen").

Without intending to be limited by theory, there are three principal phases of the hair growth cycle: anagen, catagen, and telogen. It is believed that there is a longer telogen period in C3H mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) from approximately 40 days of age until about 75 days of age, when hair growth is synchronized. It is believed that after 75 days of age, hair growth is no longer synchronized. Wherein about 40 day-old mice with dark fur (brown or black) are used in hair growth experiments, melanogenesis occurs along with hair (fur) growth wherein the topical application of hair growth inducers are evaluated. The Telogen Conversion Assay herein is used to screen PGF's for potential hair growth by measuring melanogenesis.

Three groups of 44 day-old C3H mice are used: a vehicle control group, a positive control group, and a test PGF group, wherein the test PGF group is administered a PGF used in the method of this invention. The length of the assay is 24 days with 15 treatment

days (wherein the treatment days occur Mondays through Fridays). Day 1 is the first day of treatment. A typical study design is shown in Table 3 below. Typical dosage concentrations are set forth in Table 3, however the skilled artisan will readily understand that such concentrations may be modified.

Table 3 - Assay Parameters

Group #	Animal #	Compound	Concentration	Application volume	Length of Study
1	1 - 10	Test Compound	0.01% in vehicle**	400 µL topical	26 days
2	11 - 20	Positive Control (T3)*	0.01% in vehicle**	400 µL topical	26 days
3	21 - 30	Vehicle**	N/A	400 µL topical	26 days

* T3 is 3,5,3'-triiodothyronine.

**The vehicle is 60% ethanol, 20% propylene glycol, and 20% dimethyl isosorbide (commercially available from Sigma Chemical Co., St. Louis, MO).

The mice are treated topically Monday through Friday on their lower back (base of tail to the lower rib). A pipettor and tip are used to deliver 400 µL to each mouse's back. The 400 µL application is applied slowly while moving hair on the mouse to allow the application to reach the skin.

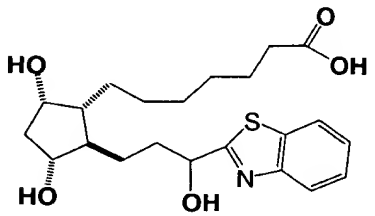
While each treatment is being applied to the mouse topically, a visual grade of from 0 to 4 will be given to the skin color in the application area of each animal. As a mouse converts from telogen to anagen, its skin color will become more bluish-black. As indicated in Table 4, the grades 0 to 4 represent the following visual observations as the skin progresses from white to bluish-black.

Table 4 - Evaluation Criteria

Visual Observation	Grade
Whitish Skin Color	0
Skin is light gray (indication of initiation of anagen)	1
Appearance of Blue Spots	2
Blue Spots are aggregating to form one large blue area	3
Skin is dark blue (almost black) with color covering majority of treatment area (indication of mouse in full anagen)	4

Example 1

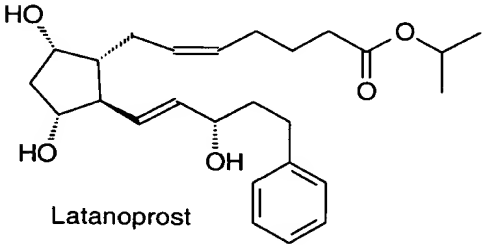
13,14-dihydro-15-(2-benzathiozolyl) pentanor Prostaglandin $F_{1\alpha}$, having the structure:



was tested according to the method Reference Example 1. 13,14-dihydro-15-(2-benzathiozolyl) pentanor Prostaglandin $F_{1\alpha}$ grew hair and had IC_{50} of 45nM.

Comparative Example 1

Latanoprost, having the structure:



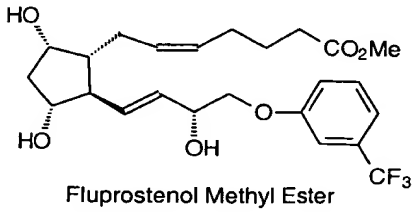
Latanoprost

was tested according to the method Reference Example 1. Latanoprost was active at 0.01% and 0.1%. Grades representing the average animal score on day 26 are reported in Table 5.

However, latanoprost is nonselective. Although latanoprost does not negate the effect of activating the FP receptor, latanoprost also activates the EP_1 receptor, which which results in the side effect of causing pain.

Example 2

Fluprostenol Methyl Ester having the structure:



Fluprostenol Methyl Ester

was tested according to the method Reference Example 1. Fluprostenol grew hair at 0.01% and 0.1%. Grades representing the average animal score on day 26 are reported in Table 5.

Table 5 - Grades

Example	PGF	0.01%	0.1%
Comparative Example 1	latanaprost	0.71	2.9
Example 2	fluprostenol methyl ester	3.9	2.6

Comparative Example 2

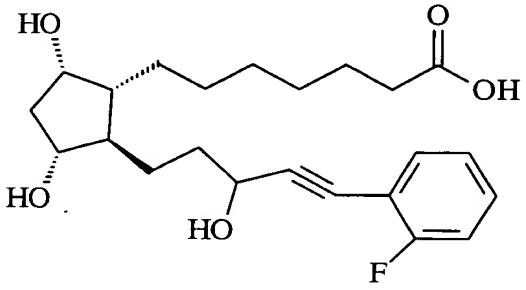
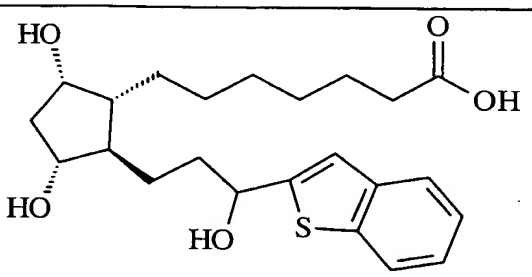
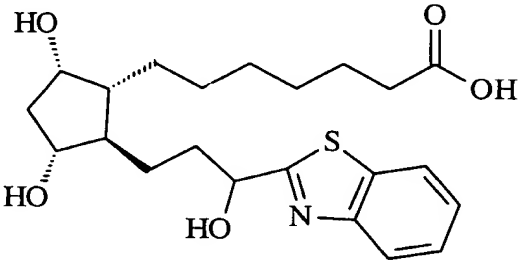
A composition containing 0.01% of a T3 compound was prepared and tested according to the method of Reference Example 1. The T3 compound grew hair.

Example 3

Compositions for topical administration are made, comprising:

Component	3-1	3-2	3-3
PGF (wt %)	0.019	0.027	0.045
IC_{50} the PGF (nM)	19	27	45
Ethanol (wt %)	59.988	59.983	59.973
Propylene Glycol (wt %)	19.996	19.995	19.991
Dimethyl Isosorbide (wt %)	19.996	19.995	19.991

The PGFs in the compositions are as follows:

Sample	PGF
3-1	
3-2	
3-3	

A human male subject suffering from male pattern baldness is treated by a method of this invention. Specifically, for 6 weeks, one of the above compositions is daily administered topically to the subject to induce hair growth.

Example 4

A composition for topical administration is made according to the method of Dowton et al., "Influence of Liposomal Composition on Topical Delivery of Encapsulated Cyclosporin A: I. An *in vitro* Study Using Hairless Mouse Skin", S.T.P. Pharma Sciences, Vol. 3, pp. 404 - 407 (1993), using a PGF in lieu of cyclosporin A and using the NOVASOME® 1 (available from Micro-Pak, Inc. of Wilmington, Delaware) for the non-ionic liposomal formulation.

A human male subject suffering from male pattern baldness is treated each day with the above composition. Specifically, for 6 weeks, the above composition is administered topically to the subject.

Example 5

Shampoos are made, comprising:

	Ex. 5-1	Ex. 5-2	Ex. 5-3	Ex. 5-4
Component				
Ammonium Lauryl Sulfate	11.5 %	11.5 %	9.5 %	7.5 %
Ammonium Laureth Sulfate	4 %	3 %	2 %	2 %
Cocamide MEA	2 %	2 %	2 %	2 %
Ethylene Glycol Distearate	2 %	2 %	2 %	2 %
Cetyl Alcohol	2 %	2 %	2 %	2 %
Stearyl Alcohol	1.2 %	1.2 %	1.2 %	1.2 %
Glycerin	1 %	1 %	1 %	1 %
Polyquaternium 10	0.5 %	0.25 %	-	-
Polyquaternium 24	-	-	0.5 %	0.25 %
Sodium Chloride	0.1 %	0.1 %	0.1 %	0.1 %
Sucrose Polyesters of Cottonate Fatty Acid	3 %	3 %	-	-
Sucrose Polyesters of Behenate Fatty Acid	2 %	3 %	-	-
Polydimethyl Siloxane	-	-	3 %	2 %
Cocaminopropyl Betaine	-	1 %	3 %	3 %
Lauryl Dimethyl Amine Oxide	1.5 %	1.5 %	1.5 %	1.5 %
Decyl Polyglucose	-	-	1 %	1 %
DMDM Hydantoin	0.15 %	0.15 %	0.15 %	0.15 %
PGF having IC_{50} of 19 nM	-	0.019 %	0.019 %	-
PGF having IC_{50} of 45 nM	0.045 %	-	-	0.045 %
Minoxidil			3 %	2 %
Phenoxyethanol	0.5 %	0.5 %	0.5 %	0.5 %
Fragrance	0.5 %	0.5 %	0.5 %	0.5 %
Water	q.s.	q.s.	q.s.	q.s.

The PGF having IC_{50} of 19 nM is the same as that in Example 3-1.

The PGF having IC_{50} of 45 nM is the same as that in Example 3-3.

A human subject suffering from male pattern baldness is treated by a method of this invention. Specifically, for 12 weeks, a shampoo described above is used daily by the subject.

Example 6

A mascara composition is prepared. The composition comprises:

Component	% W/W
WATER, DEIONIZED, USP	q.s
BLACK 1080 MICRONIZED TYPE	10.000
GLYCERYL MONOSTEARATE (2400 TYPE)	8.500
C18-36 ACID TRIGLYCERIDE	5.500
STEARIC ACID, TRIPLE PRESSED, LIQUID	4.000
ETHYL ALCOHOL SD 40-B, 190 PROOF/SERIAL #:	4.000
BEESWAX WHITE, FLAKES	3.250
SHELLAC, NF	3.000
LECITHIN, GRANULAR (TYPE 6450)	2.500
TRIETHANOLAMINE 99% - TANK	2.470
PARAFFIN WAX	2.250
PARAFFIN WAX 118/125	2.250
CARNAUBA WAX, NF	2.000
POTASSIUM CETYL PHOSPHATE	1.000
PHENOXYETHANOL	0.800
OLEIC ACID NF	0.750
DL-PANTHENOL	0.350
PVP/VA COPOLYMER	0.250
METHYLPARABEN, NF	0.200
DIAZOLIDINYL UREA	0.200
SIMETHICONE	0.200
ETHYLPARABEN NF	0.150
PENTAERYTHRITYL HYDROGENATED ROSINATE	0.150
PROPYLPARABEN, NF	0.100
TRISODIUM EDTA	0.100
PGF having IC_{50} of 19 nM	0.019

The PGF is the same as that used in Example 3-1.

A human female subject applies the composition each day. Specifically, for 6 weeks, the above composition is administered topically to the subject to darken and thicken eyelashes.

Example 7

Pharmaceutical compositions in the form of tablets are prepared by conventional methods, such as mixing and direct compaction, formulated as follows:

Ingredient	Quantity (mg per tablet)
PGF	0.5
Microcrystalline Cellulose	100
Sodium Starch Glycollate	30
Magnesium Stearate	3

The PGF is the same as that used in Example 3-3.

The above composition is administered orally to a subject once daily for 6 to 12 weeks to promote hair growth.

Example 8

Pharmaceutical compositions in liquid form are prepared by conventional methods, formulated as follows:

Ingredient	Quantity
PGF	0.1 mg
Phosphate buffered physiological saline	10 ml
Methyl Paraben	0.05ml

The PGF is the same as that used in Example 3-3.

1.0 ml of the above composition is administered subcutaneously once daily at the site of hair loss for 6 to 12 weeks to promote hair growth.

Example 9

A topical pharmaceutical composition is prepared by conventional methods and formulated as follows:

Ingredient	Amount (wt %)
PGF	0.004

Dextran 70	0.1
Hydroxypropyl methylcellulose	0.3
Sodium Chloride	0.77
Potassium chloride	0.12
Disodium EDTA (Edetate disodium)	0.05
Benzalkonium chloride	0.01
HCL and/or NaOH	pH 7.2-7.5
Purified water	q.s. to 100%

The PGF is the same as that used in Example 3-3.

The above composition is administered ocularly to a subject once per day for 6 to 12 weeks to promote eyelash growth.

Effects of the Invention

The compositions and methods herein provide a cosmetic benefit with respect to hair growth and appearance in subjects desiring such treatment.